

nature

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Risk adviser: mission impossible?

AN idea that has received wide publicity in the past week or two has been the concept of an independent community risk advisory service. Professor Gordon Atherley, of the University of Aston in Birmingham, presented the idea to the Council for Science and Society's meeting on acceptable risk and proposed that the council should take some initiatives itself to establish such a service. For its part, the council, in its forthcoming report on the acceptability of risks, commends the idea—'only in such a way can there develop an involvement of people experiencing risks, which is so necessary for fair decisions and effective control'.

There are many attractions to the idea. Professor Atherley envisages the scheme being staffed by a small band of volunteers with a scientific training who would agree to attend 'clinics' regularly in local communities to listen to and advise on problems connected with risk. The council suggests a full-time paid adviser, using part-time help. The concept would certainly be moving with the social tide, which is undoubtedly flowing away from monolithic centralised organisations towards local, more informal bodies. And analogies can be found, for instance in community law centres set up to help those who might be overawed by the legal establishment or its fees. The intention is that in the main the adviser would act as a source of information, maybe doing a little monitoring or research himself or herself. The proposal is clearly thoroughly worthy, in keeping with the council's previous institutional suggestions. But the question is, is it workable?

It is certainly going to tread on a few toes. The relationship of 'risk' to pollution, infectious diseases, safety at work, hygiene, public order, nuclear safety, fire and explosive hazards and road and rail safety is going to be hard to define. In all of these there are already deeply entrenched interests working at local or national level; it is certainly not clear that such interests, many of which have an excellent if unpublicised track record, will take at all kindly to a new figure in the community. This need be no bad thing if the community risk adviser were able to pursue an independent course of research, but this is most unlikely—in many matters the adviser will have to depend on these very organisations for data and even interpretation. Can this be done without the generation of ill-will or corruption? Of course a risk adviser would

ideally be on the lookout for new risks for which no control mechanism yet existed and might, for instance, have some success in the field of noise; but in most other fields he or she might need almost extra-sensory perception to foresee the extraordinary ways in which risk appear.

However attractive the idea of spreading information about risks around the community in the same way that a legal service answers questions about the law and a consumer bureau provides ratings of relative value for money, there is a danger that the function of a specifically risk-oriented agency would be misunderstood as a place where answers would be given. And then, of course, the community would quickly turn on an adviser whose subjunctive-ridden replies had been misunderstood as authoritative pronouncements and proved wrong by later events. Would this make for a fulfilling job for a community risk adviser? It is perhaps in questions about the calibre of a person who would do such a job that we have the most serious reservations. Unpaid volunteers, says Professor Atherley; £3,500 per year, says the council—it is difficult to be serious about a scheme relying so much on voluntary involvement or modest salaries. The community risk adviser would have to be such a paragon of virtue, so widely knowledgeable, so unswayed by pressure groups, so diplomatic in dealing with toe-trodden agencies, so tough in living with decisions, that it is doubtful that many likely candidates exist.

That a community risk adviser may not be the way to go does not, however, close the issue. The social tide is still flowing and the public is bound to want to know more and not less about hazards and nuisances. An immense amount of expertise already exists in the existing agencies and it seems desirable that before we set up yet another we should look very carefully at how well the present agencies present themselves to the public. It is as yet by no means clear whom one should write to, phone or go and see when worried about potential or actual hazards. More informative telephone directories, advertising on television and in newspapers would all help to encourage the public to take a less Kafkaesque view of governmental agencies. If in a few years there was no growing sense of public participation in coming to terms with risks, then it might be time to try and set up a risk advisory service. But let the present agencies show their paces first. □



International science (1): NATO

Plain-clothes science

Chris Sherwell, recently at NATO,
looks at the alliance's science programme

WHAT is NATO? To most people it is simply the western alliance, the collection of fifteen nations formed after the Second World War for the purposes of mutual defence. Others see it as much more. For instance, when Jimmy Carter delivered his keynote address at the recent NATO summit in London, he saw the alliance as political and economic as well as military—which of course it is.

Indeed, NATO has always been these things and more. So broad is it that, to the amazement of many western scientists, it stretches as far as a civilian science programme. In the vast concrete labyrinth of buildings and corridors that are NATO's European headquarters in Brussels, there is a handful of offices occupied by a friendly group of what in other continental circles are usually called 'committed Europeans' because of their internationalism. There are just over half a dozen of them, and they discharge responsibilities created almost a decade after the North Atlantic Treaty was signed in 1949.

That treaty, which is most explicit about the threat of war and the resolution of disputes, contains in Article 2 vaguely worded sentiments about a better understanding of the principles upon which free institutions are founded. At the end of 1956 a committee of three foreign ministers—Lester Pearson (Canada), Gaetano Martino (Italy) and Halvard Lange (Norway)—produced a report on non-military cooperation. Like the devil and sin, they were for it; in particular they argued that science and technology was "one area of special importance to the Atlantic community".

From this and further suggestions by these so-called 'Three Wise Men' for recruiting and fostering contact between scientists, was born a Task Force on Scientific and Technical Cooperation. That was in July 1957. Then, fortuitously, came the single most important event to ensure the future of a NATO involvement in civilian science: the launching of the first Soviet Sputnik in October 1957. Two months later the Task Force reported its findings to the heads of government. Its recommendations were predictable.

The future of the West, the Task Force proclaimed, was dependent to an ever-increasing degree on the rate at which science and technology advanced. It was therefore in NATO's

interest to do all it could "to bring about the general conditions which will accelerate the pace of scientific progress"; deficiencies in science and technology should not be allowed to hamper the effort to strengthen NATO members' economies and defences.

Committee plus adviser

That was the reasoning, and it was accepted. Institutionally it produced NATO's Science Committee and a Science Adviser to the NATO Secretary General who was soon called Assistant Secretary General for Scientific Affairs. He is now Professor M. Nimet Özdas from Turkey, a mechanical engineer by training; he has occupied the position since 1973 and is the eighth holder of the post. Among other things, he chairs the Science Committee, on which sits one representative for each country (the SRC head, Sir Sam Edwards, represents Britain, for example); it met for the first time in March 1958 and, unsurprisingly, there are plans afoot for a Commemoration Conference next spring to mark NATO's 20 years in the business. What form that will take remains unclear as yet. What business NATO has been in is available for anyone to see.

One business it is not in with its science programme is defence-related research, save in a most indirect and thus unimportant sense. That activity falls under a separate division of NATO altogether. Because of his position Özdas is aware of work being done, but it has little to do with the nuts and bolts of his science programme. Özdas does say the security of the alliance is important as a purpose behind NATO's science programme, but then he could hardly be expected to say otherwise. The programme's direct impact is confined largely to the scientific community it seeks to help. Indeed, a cursory examination of the work supported and the cushioned impact it has outside scientific circles suggests that NATO's involvement in science is as benign as that of practically any other funding agency.

If there is any keynote, it is collaboration. Next to publication, collaboration is seen as the essential ingredient for the progress in modern research that is NATO's expressed concern. But NATO is anxious that it should do things which aren't already done and that this should happen

without at the same time adulterating in any way the established standards that ensure quality work.

It is admitted that the latter has not always been achieved—that in earlier days mistakes were made and NATO could have acquired a reputation for being a 'soft touch'. But its peer review system has been tightened up in recent years. If charmed circles of researchers who know the system extract money more easily than others, it isn't easy to prove. The system is open to anyone, and the criteria are clear. Where there is abuse, the only sanction at NATO's disposal is to refuse money the next time round.

Altogether, six fragments make up NATO's efforts to stimulate scientific cooperation. A Science Fellowships Programme, coordinated by Bulent Bayraktar, who is from Turkey, consumes the bulk of the Science Committee budget of some \$9 million. In this field Özdas believes that NATO is unique. No other organisation, international or otherwise, is funding the international exchange and foreign training of young postgraduate and postdoctoral students on such a scale (500–600 fellowships a year).

The focus of the second biggest consumer of funds, the Advanced Study Institutes (ASI) Programme, is higher up the academic ladder. Again emphasising international aspects and seeking to do something unique, the programme aims to provide high-level tutorials and seminars on selected topics, usually of an inter-disciplinary character, on the basis that such matters might not otherwise be covered and the people involved in them might not otherwise come together.

NATO funds some 50–60 applicants a year who are themselves responsible for organising the ASI. Each ASI usually lasts up to a couple of weeks and can involve as many as 30 lecturers and 100 participants. The coordinator of the programme from Brussels, Tilo Kester, a German, receives applications each year running into hundreds which must be refereed after initial screening. The \$30,000 or so that each ASI costs goes entirely on travel and living expenses; the direct benefit gained by the organiser is chiefly in the form of a publication.

International collaboration between scientists is further encouraged by the Research Grants Programme. International collaboration, in fact, is the *sine qua non* for participation in the programme, which offers financial aid to joint projects that involve laboratories and institutes in different countries. What NATO tries to do is fill the gaps left in respect of travel by national grants.

Some 75% of the money disbursed goes on living and travel expenses, and

the awards, which are also determined through peer review, are now of the order of \$4,000 where they were once up in the \$30,000 bracket. Demand has increased in recent years, and the programme's coordinator, Mario di Lullo from Italy, insists that the prospect of publishing either one or several papers is not a passport to a successful application, even though publications are expected to have a NATO credit.

Two relatively minor activities funded out of the Science Committee budget are the Senior Scientists Programme, which awards a small number of senior fellowships and visiting professorships each year, and the Science Committee Conference Programme, which, unlike other programmes, is organised directly from Brussels. Less than a dozen of these special conferences on particular topics have been held.

The sixth fragment of NATO's science activity is intentionally the least general but it is no less complex to administer. It breaks down into six parts. Known collectively as Special Science Programmes, they complement the other programmes and are designed to ensure coverage of specific topics. These include marine science, eco-science, air-sea interaction (a meteorology programme ended in 1972), materials science, systems science and behavioural science.

Seventh fragment

Özdas has another important position. This stems from a decision taken at NATO's twentieth anniversary meeting of heads of government in 1969 to create the Committee on the Challenges of Modern Society (CCMS). Özdas chairs this committee, which operates in an interesting way to avoid expensive, secret and centralised direction.

The CCMS has no budget. If any study is to be conducted, there has to be a pilot country or countries to shoulder the responsibility, probably with other countries as 'co-pilots'. They provide the funding, and they do the study. It is carried out on the basis of information readily available, with a short time horizon in mind. And for the most part it is conducted in an open fashion with participation not just by interested organisations within the alliance countries but also by countries outside the alliance—including the USSR and East Europe.

The CCMS proposes, the NATO Council approves. Eight studies have been completed and another eleven are under way. Topics include pollution, disaster assistance, health care, energy and so on.

Özdas is assisted in the discharge of his duties by Dr Philip Hemily, an American. A man sincerely concerned to know current trends in thinking

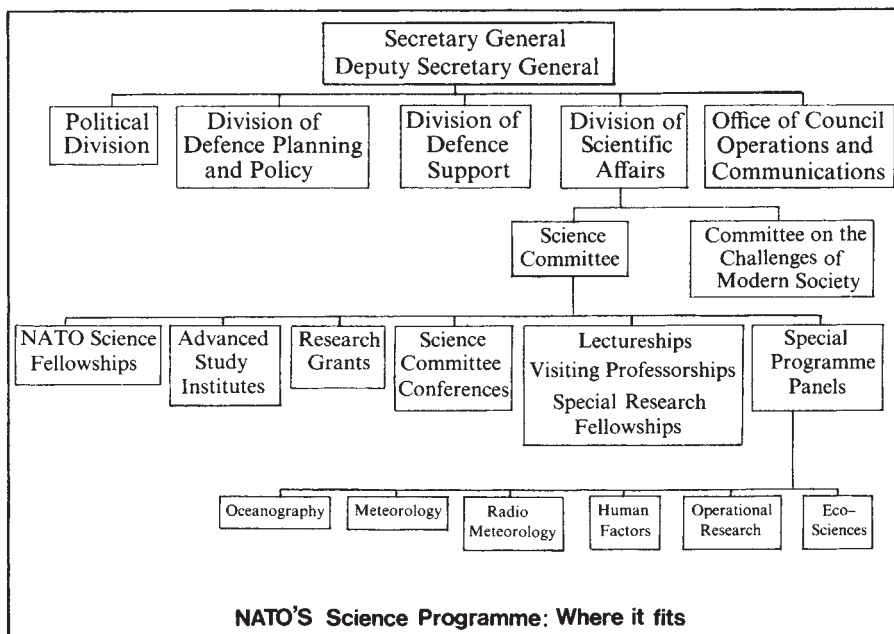


Table 1 NATO Science Committee budget (Belgian francs $\times 10^6$)¹

	1972	1973	1974	1975	1976	1977	% breakdown
Fellowships	135	137.7	144.0	148.0	160.0	172.0	54.5
Advanced Study Institutes	46.5	50.0	60.0	55.0	61.0	72.0	20.8
Research Grants	30.5	34.5	35.0	36.0	39.0	45.0	13.3
Programme Development Fund	29.5	33.0	28.0	34.0	30.0	31.0	11.4
Total	241.5	255.2	267.0	273.0	290.0	320.0	—

¹£1 = BF 62.4, \$1 = BF 36.3; all figures in current terms.

Table 2 Distribution of funds (%)

	Overall budget	Science fellowships (1960-72)	Research grants (1960-72)
United States	24.20	12.63	10.8
United Kingdom	19.50	13.96	17.2
France	17.10	11.98	6.2
West Germany	16.10	13.43	5.8
Italy	5.96	15.00	18.0
Canada	5.80	4.90	3.9
Belgium	2.86	2.74	3.8
Netherlands	2.85	2.62	3.3
Denmark	1.65	1.60	2.0
Turkey	1.65	9.65	11.0
Norway	1.15	1.57	3.7
Portugal	0.65	4.40	5.1
Greece	0.39	4.20	8.0
Luxembourg	0.09	0.26	0.0
Iceland	0.05	0.25	1.5
NATO (Adm Exp)	—	0.81	—

about contemporary problems, he is realistic enough to know the limits of NATO's capacity to help solve them. Of both the virtues and pitfalls of NATO's involvement in science he and his colleagues are only too aware. But they remain committed to the ideals which inspire scientific research, and see a role for NATO.

Thus, asked the obvious question, "Why NATO?", their immediate response is equally obvious: "Why not?" Pressed further, they steer clear of

arguments about common defence and security, preferring to fall back on another point: that there are no apparent alternatives available. No other international body is apparently working to oil the wheels of scientific research by providing finance to encourage international collaboration. That becomes their most positive argument: in the jargon of the men at NATO, they are 'action oriented'. And there is no doubt in their minds that the action is needed. □

International science (2): OAS

Science for progress

Mahindra Naraine traces the history of the OAS's involvement in science in Latin America

THE Organisation of American States (OAS) is probably the least well known of the major international organisations. United States hegemony over the nations in the western hemisphere has resulted in scant attention being paid by 'outsiders' to the OAS and its operations. Yet it is interesting that the OAS is the oldest international organisation of its kind in the world. Not only that; for the past ten years it has also had a Scientific and Technological Programme. With a budget in 1976 of about \$10 million (about 20% of the OAS budget), this programme supports such work as the development of solar refrigeration in Mexico, the study of fur seals in Uruguay, computer technology in Brazil and deforestation studies in Haiti. In comparison with this the science and technology efforts of some other organisations seem puny.

Although the OAS has been in existence for some time its main pre-occupation has been the idea of hemispheric peace, naturally under the guiding hand of the United States. The Cuban revolution and the Kennedy Administration together helped to speed up efforts aimed at the economic sector. The much-acclaimed Alliance for Progress, set in motion in August 1961 at Punta del Este (Uruguay), had the intention of eradicating economic under-development in Latin America. By the late 1960s corruption and maladministration minimised the potential benefits of the influx of some \$1,000 million into the region. The programme did not work well, and by 1967 it was obvious that a political boost to the OAS was necessary.

The American Chiefs of State met that year and in the 'Declaration of Punta del Este' stated that their aim was the creation of a Latin American Common Market. This was to begin in 1970 and to be in operation no later than 1985; it was to be a convergence of the then existing Latin American Free Trade Association and of the Central American Common Market system. The declaration also expressed the intention of harnessing science and technology for the service of the Latin American peoples. To effect this it was decided to establish a Regional Scientific and Technological Programme (PRDYCT). It was also stated that because of the magnitude of the

investments required for science and technology, it was necessary to have inter-American cooperation.

In compliance with the Punta del Este Declaration, the Inter-American Cultural Council formally established PRDCYT in February 1968. In this Resolution of, Maracay it was stated that PRDCYT

... shall be oriented towards the adoption of measures to promote scientific and technological research, teaching and information, basic and advance training of scientific and technical personnel, and exchange of information. It shall intensively promote the transfer to and adaptation by the Latin American countries of knowledge and technology originating in other parts of the world.

PRDCYT was not the first programme of a technical nature to exist in the OAS. As far back as 1950 a programme of Technical Cooperation was created to deal with requests for technical assistance. With the advent of the Alliance for Progress there was a rapid expansion of technical assistance projects. The OAS then established a special training programme in which, for the first time in OAS history, countries outside the hemisphere could participate. Several European countries participated, but the most significant contribution in this category has come from Israel. In 1975, for instance, Israel provided 70% of the experts for technical co-operation from non-member countries. Mechanisms for the operation of

PRDCYT were somewhat predetermined by the imperatives of integration and infrastructure. PRDCYT set out to create centres of excellence for the training of personnel from all the member countries. Three mechanisms were involved: multinational projects, supporting actions and basic studies.

Multinational projects are activities that are carried out in one or more centres in the region with the cooperation of several or all member states. Activities could be of a regional, sub-regional or national character. Regional activities are carried out in selected centres and in general consist of special courses, seminars and other types of technical meetings. Activities within the national sphere are meant for institutional development and to improve research and education. The OAS identified two kinds of centres: a responsible centre operated as headquarters for regional activities and provided assistance at a national level to less well endowed centres; centres that received these benefits were called participating centres.

For the less well endowed centres 'supporting actions' were expected to strengthen the scientific and technological infrastructure by building up equipment and other resources. The third mechanism, basic studies, was meant to obtain information on the region's scientific and technological capability and development.

Main emphasis

In the early years of PRDCYT the main emphasis was on multinational projects. These were allocated about 60% of the budget, in keeping with the big push for Latin American integration. There were multinational projects on mathematics, physics,

A union of American Republics has existed since 1890 but it was only in 1948 that a Charter was drawn up and the Organisation of American States created. The OAS consists of 25 member countries spread over two continents and six islands in the Caribbean. Cuba still remains a member but in 1962 was excluded from participation in OAS affairs.

Early attempts were made by Simon Bolivar to develop the ideal of regional solidarity, but these failed. It was not until the Monroe Doctrine and the idea of the Western Hemisphere as the region of United States influence that hemispheric unity, even if somewhat unbalanced, was feasible. The OAS has therefore suffered the criticism that most of its actions have been for the benefit of the dominant partner—the US.

OAS headquarters are in Washington; 18 of the member countries are Spanish-speaking. Other related OAS organisations include:

The Pan American Health Organisation (PAHO). Based in Washington, this was founded in 1902 and promotes and co-

ordinates hemispheric efforts to combat disease, lengthen life and promote health in general. PAHO also serves as a regional agency of WHO and maintains close relations with the national health services in member countries. In 1974–75 its budget was \$34 million.

The Inter-American Institute of Agricultural Sciences (IICA). Founded in 1942 with headquarters in Costa Rica, it is the specialised unit of the OAS for agriculture and does teaching, research, technical assistance and conservation. Its activities are spread over the four geographic zones of the region. IICA manages the Simon Bolivar fund set up for rural development.

The Inter-American Nuclear Energy Commission (IANEC). This is a technical commission set up in 1959 to serve as a centre of information and consultation for the member states and to offer them the necessary cooperation and assistance for the peaceful uses of nuclear energy. The commission is financed mainly by funds from the regular budget of the OAS.

chemistry, genetics, biochemistry and so on. Not all the countries benefited equally from the project, however. The more developed countries had had a greater exposure to science and made more use of the new opportunities.

The main criticism, however, came from Latin American academics, mainly social scientists. Continued pressure resulted in the OAS sponsored 'Conference on the Application of Science and Technology to Latin American Development' (CACTAL). This conference was held in 1972 in Brazilia and its recommendations, called the Consensus of Brazilia, had a major impact.

Essentially CACTAL posited that development of a scientific and technological infrastructure that was not very closely linked to the productive sector was fruitless. What was important was that national science policies be closely coordinated with economic and social policies. There must be removal of technological dependence on the developed nations, indigenous technologies had to be developed, teaching and research should be geared to the labour market and the productive sector, scientific and technological development should give preference to the rural and urban marginal sectors and national science policies should look at such matters as patents, trademark legislation and technology transfer agreements.

The OAS noted the resolutions and attempted to modify its programme. The OAS 'Mar del Plata Resolution' acknowledged that the structure of PRDCYT was too rigid to meet the demands of the member states in a timely manner. It recommended that more attention be paid to economic and social development; for this there would be a revision of the fields of action and types of activities. It was agreed that there was an urgent need for scientific and technological development in the developing countries; for this a special Mar del Plata fund was created giving preference to them.

Funding

The nature of the funding of OAS activities is somewhat complex. There is an OAS general fund to which all members contribute according to quotas established by the OAS secretariat. The total budget for 1961 was \$8.3 million. In 1968 it was \$17.2 million and in 1975 \$56.5 million.

PRDCYT is administered by the Permanent Executive Committee of the Inter-American Council for Education, Science and Culture (CEPCIECC). CEPCIECC works out a budget for its fiscal period in line with previous years and proposed projects. Members are then asked to

pledge amounts to this CEPCIECC fund in a similar ratio to the OAS general fund. Contributions are voluntary. Typical ratios are 0.19% for the poorest countries, 7.4% for countries like Brazil and Argentina, and 66% for the United States.

The CEPCIECC budget is for science, education and culture. Until 1974 PRDCYT was allocated 60% of the budget but this figure has since been reduced to 50%. It has been said that the change is a consequence of most of the representatives on CEPCIECC being Ministers of Education. In its first year of operation PRDCYT was allocated \$2.4 million excluding administration costs. In the first three years the programme disbursed some \$18 million. For the next five years over \$50 million was spent.

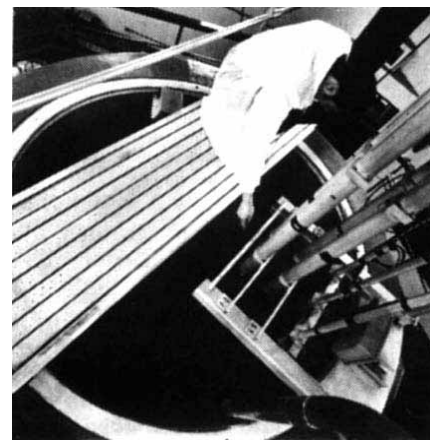
The funding has been further complicated by the creation of the Mar del Plata fund in 1974. Under this each country has a multiplier factor; the poorer the country, the higher the multiplier. Countries whose quota to the general OAS fund is less than 0.75% have a multiplier of about 7; for a country like Mexico it is 1.9. A country then allocates money to this special fund providing it does not exceed the sum it contributes to the regular fund. This contribution is then increased by the multiplier factor; the matching funds are made up mainly by the United States. For the 1974-76 period the Mar del Plata account had some \$5.8 million.

At the present time PRDCYT acts in areas of basic and applied sciences (supporting the funding of research projects and training of personnel), technological research and the transfer of technology. It is also involved in scientific and technological policy and planning—helping in the formulation of national science policies.

Applications for support are sent through the national science agencies to the OAS. The OAS has a panel of experts which provides technical guidance on the application. Projects can request training of personnel, recruitment of visiting professors, equipment, books and so on, but do not cover administration expenses or other requirements. In compliance with political attempts at integration, however, preference is given to projects that are multinational, so that proposals always include provision for contact or assistance with other regional institutions or personnel.

Main problem

Ironically, PRDCYT's main problem has been its association with the OAS. The programme was developed through political actions and it has never lost that association in the eyes of its critics. The United States' dominance



Bolivian reactor project

of the OAS and its manipulation of the region to secure hemispheric solidarity has raised many doubts about the worth of any OAS programme.

Undoubtedly, the benefits have not been as large as they might have been. The more developed countries have benefited most from the programme, but this should be expected since they have a better science and technology infrastructure. The Mar del Plata account has helped to redress the balance, but the main issue here is that it limits the amount of money countries can obtain. The newly created Simon Bolivar fund, financed mainly by Venezuelan petrodollars, will concentrate its efforts on rural development—PRDCYT's main problem is that it has not been closely linked to direct economic development. Criticisms of it on technology transfer conditions, patent legislation and types of development, however, are spurious, since these are issues that have to be settled internally by national policies. Awareness of these problems on the part of some national agencies has actually stemmed partly from PRDCYT-organised courses, but these agencies find it difficult to convince their superiors of the need for integrated science policies.

PRDCYT has been important in the development of the scientific and technological infrastructure of the Latin American nations. Through its efforts many centres of excellence now exist that are the prerequisites for the generation of indigenous technology. Many institutions rely on the programme for financial support for research, since they receive very little money directly from their own governments. According to Article 45 of the OAS Charter, governments should encourage science that is oriented toward the overall improvement of the individual—that is a foundation for democracy, social justice and progress. In too many Latin American countries money is used for the very opposite purposes. □

Danger: men at work

Judy Redfearn looks at the problems facing those concerned with major hazards in Britain

ALMOST three years ago an explosion at a Nypro factory in Flixborough killed 28 people and injured more than 100. It shocked the British public into awareness of the possibility of other major industrial accidents. The Health and Safety Commission's Advisory Committee on Major Hazards was set up in the wake of Flixborough to lay down recommendations for the control and supervision of major hazards. Nine months ago it published its first report, which helped those concerned with industrial safety to think seriously about its recommendations and how to implement them. Some of the problems that report brought to light have since been discussed, notably at a meeting organised by the Institution of Mechanical Engineers at the beginning of May, when representatives from industry, local authorities, the Health and Safety Executive (HSE) and the committee itself got together in London.

The report's main recommendation poses a major problem. The recommendation was that a 'notification' scheme should be set up to help the HSE compile a list of major hazards: all installations would be subject to review, even though most would demand no more regulation than already exists under the 1974 Health and Safety at Work Act; but potentially hazardous installations would be required to submit a detailed survey of their activities to the HSE. At the moment, this is voluntary, but the committee would like to see it enforced by legislation.

The problem with the scheme is the burden it will impose on the HSE. Not only will the Executive receive a large number of surveys from all kinds of installations; it will also need to examine in great depth those which might indicate a major hazard. To relieve some of this burden, the committee has suggested that individual companies should be responsible for indicating their own problems and outlining the solutions. But this still means the HSE will need more specialised staff. The committee has suggested that outside expertise should be made available at any time.

Another difficulty is that it is not yet clear how the top league of hazardous installations would be controlled: whether by regulation or licensing. The committee feels that it can only wait until both local authorities and the HSE have acquired, under the notification scheme, fairly comprehensive in-

formation on the types of activity they will be controlling and on the problems they will be facing before it can recommend which way to go. One of the dangers it wishes to avoid is transferring liability for major accidents from industry to the regulating body. Whatever machinery is set up, some industrialists feel that a major stumbling block will come in trying to ensure that their special case is understood by whoever is to judge it. And in the end what does or does not constitute a major hazard will be someone's subjective judgment.

Nor is this all. Accidents happen—either through the fault of people operating machinery or through mechanical breakdown—but the extent of the damage they cause depends on environmental factors. The report recommended that new plants should be planned to take account of the likely consequences of a major accident: buildings should be capable of withstanding shock from explosions and of containing toxic chemical leaks and should be situated away from densely populated areas. Machinery should be designed to the highest specifications and staff should be continually retrained in operating procedures.

The trouble is, human error can never be eliminated, whether in the

design or in the operation of an industrial plant. The recent meeting produced a general call for more research into the relative safety of automatic control systems and human control: it was asked, for example, whether it was safer to leave everything to machines and to have no one about when an accident does happen, or to have somebody there to warn others when something goes wrong. The committee is also concerned about the lack of knowledge on the massive release of toxic and flammable gases that are heavier or lighter than air.

Industry expects other problems once a plant has been labelled a 'notifiable installation'. For example, people have to be convinced that it is safe to work there. The feeling is that if a reputation has been established for giving reliable information on all aspects of its work, employees are likely to trust what is said about safety. The difficulty is that too often in the past industries have been found to have wrong statistics even on fundamental points. Public confidence will only be inspired if industries are seen to be reliable. A possible way of educating the public might be through the unions, but they have yet to be approached.

The committee's work is still in its first stages. It plans to hear more evidence and produce other reports as and when it sees fit. It still has many points to clarify before it can recommend further action and through the HSE it is liaising with the Dutch—the only other nation looking into major hazards. In the meantime it is simply waiting to see what turns up. □

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Keystone

Devastation at Flixborough

ITALY

Distant drums

The pollution threat posed by the cargo of a submerged ship in the Adriatic could now be receding. Alastair Hay, recently in Italy, reports

FOLLOWING a collision with a Panamanian ship in July 1974, the Yugoslav vessel *Cavtat* sank three and a half miles off the coast of Otranto, in southern Italy. The *Cavtat* was carrying as part of its cargo 270 tons of the petrol 'anti-knock' additives lead tetraethyl and lead tetramethyl. These were stored in drums and located in the hold and on the deck. Three years later, most of the drums are still there—under three hundred feet of water at the entrance to the Adriatic.

The pollution problems of the Mediterranean are legion. Those of the Adriatic are even more numerous. Industrial effluent discharged directly into the Adriatic without prior processing has led to an increasing accumulation of heavy metals, particularly of lead, zinc, cadmium and mercury. The Italian government has taken steps to control the discharge of untreated effluent. However, equal concern was not at first in evidence for the pollution threat posed by the leakage from the drums in the *Cavtat's* cargo. Indeed, it took the government more than two and a half years to act: on 3 February this year, following a midnight cabinet meeting, the government finally announced that £6.7 million was being set aside over a two-year period for salvage operations to raise the drums.

Several factors forced the government to take this decision: a growing realisation that the cargo, if left undisturbed beyond the spring of this year, will be impossible to salvage; publicity about the pollution hazard which is having a serious effect on the local tourist and fishing industries; and a vocal Otranto magistrate, Alberto Maritati. Shortly after the accident,

Maritati began an investigation and sought scientific advice on the hazards posed by the lead compounds. Most scientists agreed that, if the chemicals were to escape from their drums, a major ecological disaster could well ensue. But opinion differed as to the advisability of raising the drums to the surface. Some considered that the drums were better left on the sea bed and perhaps carpeted with a layer of concrete. The British firm of Octel, who manufactured and packed the lead products, considered the *Cavtat* problem to be a storm in a teacup, the company's technical manager reportedly having said that "... the best thing to do is to leave the drums down here. If it was all released at once, it wouldn't cause much of a problem in my opinion. I think it is an exercise in sheer futility".

This, however, has not been the majority view of the problem. Reports from marine scientists and toxicologists confirmed Maritati in his opinion that the lead compounds were hazardous and could enter the marine food chain. He attempted to obtain government support for his decision to raise the *Cavtat's* lethal cargo. Despite dozens of journeys to Rome and many formal written requests for assistance he received little support for his project. Maritati had powers invested in him as a magistrate to initiate the salvage programme, but he had no funds to pay for it. This was the government assistance he sought. Finally, on 26 January, and still without the promise of government aid, Maritati issued an order requiring the Saipem company—experts in underwater engineering, and a subsidiary of the Italian state oil company, ENI—to begin salvage operations. The magistrate was convinced of the need for urgency after the inspection of a drum raised in December by a team of divers had revealed corrosion of the containers and confirmed their porosity; it seemed essential that all the drums be brought to the surface before further corrosion made the operation too hazardous.

Interviewed recently, Maritati told *Nature* that he required no additional assistance from the central government. A Communist Party member dealing with Christian Democrats, he said his efforts to enlist government support had resulted in an assurance that it would pay for the entire salvage operation. He was told that there is now an arrangement between the Department of the Merchant Navy and

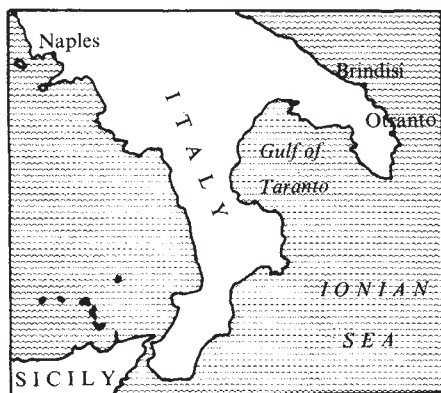
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Poisoned fish caught in the Gulf of Taranto

Saipem "that all the drums will be raised". Saipem is working with the manufacturers Octel, who will handle the drums when they are brought to the surface.

The most difficult part of the salvage process will be to secure the drums in the ship's hold. However, Saipem are convinced that the problem is not insuperable and have assured Maritati of this fact. Maritati says that it is "a problem which requires urgent attention. I visit the site every day to ensure that the operation is proceeding to my satisfaction. There should be no interruptions in the salvage operation. This being so, all the drums will be raised by June. And so tourists will have nothing to fear".

Once the cargo is neutralised the *Cavtat* problem will still feature prominently in the courts. The captain faces criminal prosecution on two counts: that of polluting territorial waters, and of criminal negligence—he is alleged to have refused the help of Italian tugs which could have towed his ship to safety. Maritati will be involved in this legal process, but for the moment he has won his case for government assistance. The government, too, is anxious to bring the matter to a speedy conclusion. Officials in the Ministry of Foreign Affairs were insistent that central government hesitation on the issue was not due to any deliberate obstruction, but to the fact that so many departments were involved in the affair; consultation with and between all the departments was essential before any action could be taken. That action is finally in view. Everyone hopes that the outcome will be a potentially cleaner Adriatic. □



USA

●A key advisory committee last week decided to recommend that guidelines which now govern recombinant DNA experiments in the United States should be amended to facilitate experiments with human DNA. Though the committee's recommendation, which will eventually be made to the Director of the National Institutes of Health (NIH), may not be accepted, it is sure to generate considerable debate.

The committee, the Recombinant DNA Advisory Committee, carries considerable weight because it was largely responsible for drafting the present NIH guidelines. The guidelines require that recombinant DNA experiments involving human genes must be performed in the highest level of physical containment (P4), and in addition, crippled strains of micro-organisms must be used (a so-called EK2 system). Those requirements effectively bar work with human genes for the time being because no P4 laboratories have yet been certified, and few universities are likely to build them. The advisory committee will recommend, however, that the containment level should be reduced to P3 and EK2, a level which would allow such work to go ahead in many places.

The committee decided to recommend the change for a variety of reasons, and its vote was 11 to 1, with one abstention. A number of committee members told *Nature* last week that they agreed to recommend the change because they feel that evidence, accumulated since the original guidelines were drafted, indicates that potential hazards associated with many types of recombinant DNA research are extremely remote, and work with human DNA is likely to yield important results. In particular, Roy Curtiss of the University of Alabama, who proposed the change, argued that so far nobody has found faithful expression of vertebrate DNA in *E. coli*, that if cloned DNA were to get into the intestine it would rapidly be degraded by enzymes in the gut, and that several experiments suggest that *E. coli* containing 'foreign' genes is at a selective disadvantage.

The move is sure to be criticised on scientific as well as political grounds, however. Experiments involving human DNA were assigned to the highest level of physical containment largely because of the possibility that human cells may harbour cancer viruses whose genes might be picked up and cloned along with the human DNA, but some of the advisory committee members noted that so far an

intensive search for human cancer viruses has failed to find any. That potential hazard cannot be entirely ruled out however, and the recommended relaxation of the guidelines is therefore likely to be controversial.

The committee will meet again in June to discuss further recommendations to amend the guidelines, and its proposals will eventually be sent to



the NIH Director Donald Fredrickson, who will almost certainly subject them to public discussion before acting upon them. In the meantime, Congress is busy working on legislation to regulate recombinant DNA experiments, and it is likely to require that new regulations be drafted. It could take as long as 18 months for new regulations to be drafted and implemented, however, so the NIH guidelines are likely to be important for some time yet.

●Meanwhile, in a move which is sending a few tremors through the biomedical research community, the environmentalist organisation Friends of the Earth, has gone to court to seek an order halting federal funding of recombinant DNA research in the United States. The organisation has charged that NIH violated the law by failing to publish an environmental impact statement before it issued its recombinant DNA guidelines last year.

The law in question, the National Environmental Policy Act, requires that government actions likely to affect the environment must be preceded by publication of an analysis setting out the potential environmental impact and discussing alternative courses of action. NIH issued its recombinant DNA guidelines in July, and it published a draft environmental impact statement six weeks later. A final version of the statement is now

under review in the Department of Health, Education and Welfare and it has yet to be published.

The chief reason why the process got out of step was that NIH didn't realise until the guidelines were in the final stages of being drafted that an environmental impact statement would be required. A long delay in issuing the guidelines would therefore have resulted if NIH had followed the letter of the law.

When the draft environmental impact statement was published in August, NIH Director Donald Fredrickson admitted that the legal process had been short-circuited, but he argued that the public interest was best served by prompt issuance of the guidelines. Recombinant DNA experiments were then proceeding under general safety rules recommended by a group of geneticists which met at Asilomar, California, early in 1975, and since the NIH guidelines are more strict than the Asilomar rules, Fredrickson argued that "the escape of potentially hazardous organisms was more likely in the absence of NIH action".

Friends of the Earth is claiming that because NIH failed to follow the procedures laid down by the National Environmental Policy Act, the guidelines were issued improperly and should be withdrawn. Federal funding for recombinant DNA experiments should then be halted, the suit demands, pending the resolution of safety issues.

NIH has 60 days to respond to the suit, and lawyers from the Department of Health, Education and Welfare met last week to discuss their response. Even if the courts find that NIH violated the letter of the law, however, they need not necessarily require a halt to the research.

●A bill to provide a major infusion of funds into earthquake prediction and into the design and construction of earthquake resistant buildings, dams and other structures, was approved unanimously by the Senate last week, and it has also been passed by a key committee in the House. The bill, which has been kicking around Capitol Hill for several years, is thus virtually certain to clear Congress in the next few weeks. It would authorise expenditure of \$55 million next fiscal year and a total of \$205 million over the next three years for earthquake research, and it directs the President to draft an implementation plan and to recommend ways to help communities prepare for earthquake warnings.

Colin Norman

COMECON

Space relations

The Comecon countries' joint space programme includes a manned mission planned for next year. Vera Rich describes preparations

THIS year, which marks the twentieth anniversary of the first Soviet satellite Sputnik 1, is also the tenth anniversary of the joint Comecon 'Interkosmos' programme. To date, the achievements of Interkosmos have amounted to the launching of 16 orbital probes and four high-altitude rockets. Last year, however, a major new development was announced: the Soviet manned space programme would be extended to include cosmonauts from the other member countries of Comecon.

The first trainees, said to be from Poland, East Germany, and Czechoslovakia, arrived early this year at the 'Star City' training centre near Baikonur, and candidates from other Comecon countries are joining them during the course of the year. As the first manned Interkosmos mission is planned for 1978, the training programme is clearly a considerably abridged version of the normal Soviet five-year course, and it is therefore not surprising that from the start it has been stressed that each joint mission will be captained by a Soviet cosmonaut.

The prospect of a fellow-citizen in orbit has done much to rekindle interest in space research throughout the Comecon bloc. A recent exhibition in Warsaw of Soviet scientific achievements included a special 'Kosmos-77' pavilion which, in the course of five weeks, attracted more than 1.2 million visitors. The Soviet 'Cosmonautics Day' festival—the anniversary of Gagarin's flight—fell during this exhibition and was celebrated with fitting honours, including a visit from Professor Roald Z. Sagadiev, Director of the Institute of Space Research of the Academy of Sciences of the USSR, and cosmonaut-pilot Viktor V. Gorbalko, captain of the recent Soyuz 24 mission.

The participation in the not-too-distant future of a Polish cosmonaut in a joint mission might well have been expected to form a major theme of the speeches on this occasion. However, Professor Stefan Piotrowski, Head of the Committee for the Investigation and Peaceful Uses of Space of the Polish Academy of Science, chose to concentrate on the special 'Kopernik 500' Interkosmos launch of April 1973, "the most important Polish space experiment to date", for which, in

honour of the Copernicus quincentenary, "almost all the apparatus and scientific programme" was produced by a Polish team. From the Soviet side, Professor Sagadiev spoke largely of such future prospects as deep-space probes to the outer regions of the solar system, orbital laboratories, and satellite monitoring of weather, crop prospects and pollution. The proposed launching order of the Comecon cosmonauts remains a closely guarded secret.

This is, of course, in accordance with Soviet practice which normally never permits the disclosure of details of a mission in advance. There is undoubted unofficial rivalry between the Comecon countries as to whose cosmonaut will blast off first, and a minor set-back in training or a last-minute indisposition leading to a change in order might lead to undesirable tensions and ill-feeling between the countries concerned.

It is noteworthy, however, that the three countries whose representatives arrived first at the training centre are those which have been most closely concerned with the unmanned Interkosmos probes, providing instrumentation and experiments for the satellites. East Germany, in fact, not only supplies electronics and telemetry systems for the Interkosmos satellites, but also in September 1976 provided a special 'multispectrum' camera for the Soviet Soyuz 22 mission.

The final goal of the Comecon bloc is total economic integration, and although the current five-year plans are temporarily playing down this aim, concentrating rather on a short-term (15–20 years) drive towards integration in certain significant areas, one of these chosen fields is instrumentation. The selection of East German, Czechoslovak or Polish equipment for Interkosmos satellites is an outcome of this policy. Since, under the complicated financial arrangements involved, it is the supplying countries who pay for the R&D, the planners might feel that priority given to the cosmonauts from these countries would be a graceful compliment to their considerable effort and outlay. It would also be a way of stilling the rumours of discontent that the other Comecon members are paying the lion's share of grandiose projects initiated by the Soviet Union and for which the Soviet Union gains the acclaim.

It should be remembered, however, that in spite of special satellite tracking ships, the Soviet Union relies heavily on ground bases in the Come-

con countries. Because of their geographical location the two least-developed members—Cuba and Mongolia—are particularly important in this respect; indeed, Cuba joined the Comecon 'Intersputnik' communications systems when the latter was first set up in November 1971, eight months before becoming a member of Comecon itself. Under the Intersputnik agreement ground stations are owned and operated by the countries in which they are sited. Although as developing countries Mongolia and Cuba are said to receive the necessary Intersputnik facilities from the Soviet Union free of charge (unlike the European members of Comecon which must bear the cost themselves), the value of the Havana and Ulan-Bator tracking bases to the Soviet space programme is not expressible in roubles, and the inclusion of Cuban and Mongolian cosmonauts in the training programme reflects a genuine contribution made by these countries and is not simply a tribute to the principle of the 'friendship of nations'.

Although the joint teams of the Interkosmos manned missions will presumably carry out the now routine geophysical and astrophysical observations, medical checks and, possibly, experiments on the effect of weightlessness on physical and chemical processes such as crystal growth, it seems unlikely that the non-Soviet cosmonauts will contribute any special knowledge or expertise which their Soviet counterparts could not have provided. Rather is it a grand exercise in public relations, to demonstrate the fraternal cooperation of the Socialist countries—and to provide for those countries a greater involvement in the Interkosmos programme than equipment on board a Soviet-built satellite or a tracking station on their soil. □

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Inspecting Interkosmos 10

Novosti

UK response

foreign uranium supplies, uranium enrichment as a source of weapons, development of international reprocessing facilities and storage of unprocessed fuel, he was apparently serving notice that the political battle to win a consensus could be drawn out.

UKAEA plans

Apparently in response to fears expressed by critics of nuclear power over the threat of plutonium diversion, the UK Atomic Energy Authority (UKAEA) is planning to demonstrate two methods of combating the problem. Speaking last week, the UKAEA chairman, Sir John Hill, said that the UKAEA was proposing to build a

finement. *Aviation Week* cites the revelations by the Soviet physicist Leonid Rudakov last summer during a tour of US fusion laboratories. Rudakov, "in response to a taunt by a western scientist", revealed that

[illegible]

The test site that is crucial to General Keegan's thesis is at Semipalatinsk near the Tibetan border. *Aviation Week* describes in detail a large building containing two steel spheres and states that since November 1975 seven tests "that may be related to development of a charged-particle beam device" have been carried out. The only evidence for the tests cited, however, is the hydrogen with traces of tritium in the upper atmosphere—which could mean almost anything.

"the USSR can convert electron beam energy to compress fusible material to release maximum fusion energy".

have not announced a major breakthrough since Rudakov's visit, there has not been one.

Another aspect of the technology *Aviation Week* touches on is accelerator design. For a land-based beam there is the perhaps insurmountable problem of propagation through the atmosphere without losing all its energy. A satellite-based beam is therefore an attractive proposition but would limit the size of the extremely powerful accelerator that would have to be carried on board. Theoretically there are several ways of decreasing the size of an accelerator; they include the idea (originating in the Soviet Union) of a collective accelerator in which the protons that will form the beam are initially surrounded by electrons which drag them along and are more easily accelerated; the electrons are then blown off to leave the beam.

This concept has received considerable attention both in the Soviet Union and in the United States but has now been largely discarded. The 'smoke ring' collective accelerator experiments at Berkeley, for example, have stopped because of enormous problems in creating a stable, sufficiently intense electron beam. Interestingly, *Aviation Week* fails to discuss any evidence of Soviet progress in that area. It seems therefore that at least one crucial component of a satellite-based beam weapon is not yet technologically feasible.

Even if General Keegan's assessment of Soviet technology in most areas is substantially right, there remains the claim of whether the ultimate aim is a beam weapon. The interesting thing about *Aviation Week's* list of technologies is that they are all equally crucial for the development of fusion by an electron or laser beam. The location that General Keegan interprets as a beam weapon test site is equally likely to be used for fusion experiments. And being first with fusion would confer a good deal more power than ownership of a decidedly cumbersome and delicate weapon.

fuel fabrication plant at Dounreay, now the site of Britain's 250 MW prototype fast reactor and of a fuel reprocessing plant. This would eliminate the need to transport plutonium once the initial charge is delivered.

The UKAEA also hopes to demonstrate the concept of a single reprocessing plant servicing a "substantial number" of reactors. The idea of irradiating reprocessed and refrabricated fuel before it is dispatched back to a reactor is apparently under consideration, but there may, according to Sir John, "be easier, more convenient ways of achieving the same objective developed in the interim".

Convention signed

Representatives of over thirty countries, including the United States and the Soviet Union, gathered in Geneva last week to sign an international convention known as Enmod which prohibits the hostile use of environmental modification techniques. The convention, which took three years to negotiate, allows research and develop-

ment on the manipulation of the environment for peaceful purposes.

ACOPS report

A 20% increase (500 to 595) in the number of pollution incidents around UK coasts between 1975 and 1976 is reported in the annual report of the UK Advisory Committee on Oil Pollution of the Sea. The report, out last weekend, expresses disappointment at the lack of progress at the UN Law of the Sea Conference, another session of which opened this week in New York.

More teaching companies

To improve the interaction between industry and universities, the UK Science Research Council (SRC) and the Department of Industry (DoI) have agreed to expand the Teaching Company Scheme over the next five years. They will share the cost equally and have made provision for a maximum of £2 million to be spent in any one year.

First proposed in 1975, the scheme was designed to introduce able grad-

uates to industry under the joint super-industrial workers, in the hope that vision of university academics and they would remain and follow careers in management. Initially, the scheme was taken up by five companies in cooperation with nearby universities and although these programmes have suffered problems it has been decided to go ahead with new ones. Two more schemes were approved last week: Lesney Products in cooperation with North East London Polytechnic, and Anderson Strathclyde Ltd in cooperation with the University of Strathclyde. The target is now twenty teaching companies by 1982.

Asbestos meetings

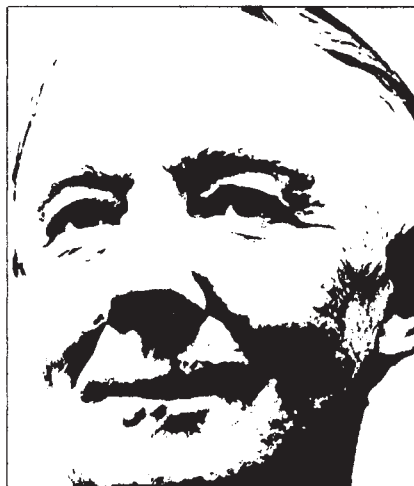
The UK Advisory Committee on Asbestos, set up in March last year by the Health and Safety Commission to review the risks to health from exposure to asbestos, has invited individuals and organisations who have submitted written evidence to attend three public meetings the committee is holding in London next month.

THE world's oil deposits are finite, and are likely to be exhausted within the next hundred years. Pessimists suggest that they may only last for twenty-five years, optimists believe there are supplies for two or three times as long. In the past oil has caused a great deal of environmental damage, including air pollution from refineries and spills from wrecked tankers such as the *Torrey Canyon*. The recent blowout at the Ekofisk rig in the North Sea is a reminder of continuing dangers which are unavoidable. It is important to ensure that this short-lived, though important, industry does not leave behind a permanently damaged world.

Although the oil industry exists to make a profit, the major companies have for some years taken a responsible attitude to the environment. A recent manifestation of this is the establishment of the Conoco Lecture by the Continental Oil Company, one of the major international entrepreneurs in this field. The first lecture was by Dr Peter Chapman, of the Energy Research Group of the Open University, on "The responsibility of industry towards the environment". He demonstrated how important it was to consider all stages in, for instance, energy generation, when trying to reduce pollution. A comparatively clean power station might depend on fuels and machinery which had already produced massive pollution. He stressed the point that

'market forces' themselves could not be relied on to protect the environment, and that industry must be prepared to accept restraints which may

Duty on oil



KENNETH MELLANBY

reduce its profitability.

Pollution control is very complicated, and few generalisations can be widely applied. Legislation may or may not be effective. The first British Alkali Act of 1867 was strongly resisted by industry, but was effective in reducing the serious damage done by hydrochloric acid from alkali works. The Clean Air Act of 1965 is generally given the credit for clean-

ing the air of London and other cities; this myth was again propounded by Dr Chapman. Actually the amount of smoke and dirt in the air in Britain had been falling rapidly for ten years before 1956, and the rate of decrease was actually slower, not faster, after the Act was passed. It would be an abuse of the statistics to suggest that this law actually prevented air pollution from being controlled, but the main causes of the improvement were economic. By 1956 much of industry had already changed from burning raw coal to using oil, gas or electricity, all cleaner (and, at the time, cheaper) fuels. The change in domestic heating had been slower, but in more prosperous areas clean methods of central heating were widely used when the Act came into force.

Since 1956 it has been possible for local authorities to declare 'smokeless zones' where the domestic use of raw coal is forbidden. This has undoubtedly made the final clean-up of such areas possible, but smoke continues to pour out of the chimneys of many houses in our poorer areas, or in those where our miners receive—and burn—over ten tons each of concessionary coal a year. The law has been much less effective than have economic forces in cleaning Britain's air. It will be interesting to see the effects on the environment of the proposed expansion of our coal industry.

correspondence

Separating swimmers

SIR,—Within the past few years the electronic timer, capable of responding to time differences of .001s, has replaced the stop-watch, capable of responding to time differences of perhaps .05s. In world class swimming events are now decided and records are established by amounts down to .01s. Detailed regulations for the use of such devices are given in the handbook of the international swimming federation FINA. A recent modification to these regulations, passed at the Montreal Olympic Congress in 1976, prohibits the separation, even for placing, of swimmers by times less than .01s. Clearly, the significance of such accurate timing needs examination.

According to FINA Regulation, a pool for an Olympic or for a World Games must have a length of 50.00 m with a "tolerance of $\pm .03$ m". In other words, the maximum permissible error in length, from one pool to another, is $\pm 0.06\%$. This error is recurrent and will remain the same, no matter how many lengths of the pool are swum. If timing is conducted to one-hundredth of a second electronically, that is to $\pm .01$ s, the maximum possible timing error in the fastest event, the Men's 100 m Freestyle swum in a typical time of 50s, is 0.02% . The timing error of 0.01 s is not recurrent, and so the percentage timing error decreases for longer distance races, taking longer times.

It is well known that the overall accuracy of any measurement is set by the least accurate measurement. For the example above, the Men's 100 m Freestyle, the maximum possible error, 0.06% , is three times the maximum possible timing error of 0.02% , the ratio becoming more unfavourable for longer distances.

It is therefore not valid to compare records made in different pools, unless the times can be corrected to the exact length of the pool, or unless the time difference is, in the case of the Men's 100 m Freestyle, at least 0.03 s and correspondingly greater for longer distances up to a maximum of about 0.5 s for the Men's 1,500 m swum in a typical time of about 15 minutes. Current listings of world records do not appear to make this distinction, and there is no indication that the records have been corrected to the appropriate pool length. Although in practice many records are established by better

margins, in some cases, especially over shorter distances, 0.01 s separation has been the deciding factor.

The figures quoted are typical and not, of course, exact. This does not alter the argument or the general conclusion, however. As surveyor's measurements are usually more accurate than the FINA tolerance of $\pm 0.06\%$, it might be better to specify all records after correction to the 'exact' (that is, the surveyor's) pool length, rather than to attempt to define separation times for each event and every pair of pools. The correction could be programmed into the electronic timer itself. In fact, it is the velocity of the swimmer that is really of interest. Time is used as a convenient indicator, but the assumption is implicit that all course lengths are all known exactly to be equal. Similar arguments may apply in other sports.

Other points might also be mentioned:

- If all the lanes in a given pool are known to be of equal length it may be valid to separate swimmers to 0.01 s accuracy.

- The well-known problem of the velocity of the sound of the starter's gun is accounted for by separate horns, one at each starting block, each linked to the timer and to the starter's gun. If this is not done, the swimmer in the farthest lane from the gun will start with a disadvantage of some 0.03 to 0.06 s, depending on the pool width and the position of the starter along the side of the pool.

- Expansion of the pool with changing water temperature has sometimes been cited as another possible source of error. Water temperature must be maintained at a minimum of 77°F and is unlikely to exceed this by more than a few degrees. For typical materials and temperature ranges the effect of expansion is about an order of magnitude smaller than the pool length tolerance and is not, therefore, significant.

Yours faithfully,

C. H. BARROW

University of the West Indies,
Kingston, Jamaica

Carcinogens and cancerogens

SIR,—The amusing exchange between Lijinsky and Jukes (7 April, page 494) appeared even more droll because they both used the term carcinogen in such a way as to leave question to their

meaning. 'Carcinogen' should refer to the production of carcinomas, that is, tumors of epithelium. Agents that produce sarcomas, leukemias or other neoplasia as well as carcinomas should properly be called cancerogens. I doubt that either correspondent intended to limit their meaning to substances producing only carcinomas—they have followed a widespread but incorrect usage.

Yours faithfully,

CECIL H. FOX

Solna, Sweden

Inexhaustible energy

SIR,—J. Tuzo Wilson (20 January, page 196) has argued that economics must change because the era of cheap energy is past. Many scientists have claimed that the watchword of the next era will be inexhaustible energy. R. W. Cahn (10 March, page 106) has given reasons why fusion power does not really meet this description.

A current campaign, in the United States at least, is to build solar energy collectors in space, and beam energy to the Earth's surface. It is said these would last as long as the Sun does. I feel there must be some flaw in this "inexhaustible energy source" too, and wish someone would tell me what it is.

Yours faithfully,

D. W. ALLAN

Toronto, Canada

Power station in the sea

SIR,—In a short note (31 March, page 402) you say that Loviisa—the location of Finland's first nuclear power station—is south of Helsinki. Have you looked at the map to see where, if this were true, it would be?

Yours faithfully,

STIG-OLOF LONDEN

Otaniemi, Finland

Molecular biology lab

SIR—There is no truth in your report (28 April, page 768) that the Governing Board of this Laboratory will be disbanded when Perutz retires in September 1979.

Yours faithfully,

M. F. PERUTZ

SYDNEY BRENNER

MRC Laboratory of Molecular
Biology,
Cambridge, UK

news and views

Photosynthesis and photorespiration

from A. L. Moore

DURING photosynthesis, many plants release previously-fixed CO_2 in a light-dependent oxidation process known as photorespiration. The process occurs chiefly in higher plants which fix CO_2 exclusively by way of the C_3 pathway of photosynthesis (the Calvin Cycle). The role of photorespiration is obscure and it is frequently regarded simply as a wasteful process; it has been proposed that net CO_2 fixation by photosynthesis could be increased by up to 50% by preventing photorespiration and the associated oxygen inhibition of photosynthesis. Since all major crop species except maize, sorghum and sugar cane actively photorespire, a major opportunity to increase crop productivity may lie in the control of photorespiration (see Zelitch *Nature* 256, 90; 1975; Chollet & Ogren *Bot. Rev.* 41, 137; 1975), and recent work by Bolhar-Nordenkampf (*Biochem. Physiol. Pflanzen* 169, 121; 1976) aimed at disentangling the complex effects of various environ-

mental factors on the rate of photorespiration and photosynthesis may be an important step towards this goal.

Factors affecting photorespiration include light, high levels of O_2 or low levels of CO_2 in the atmosphere and high temperature. Increases in temperature differentially increase the rate of photorespiration with respect to photosynthesis, whereas photorespiration increases proportionally to photosynthesis as light intensity increases. Bolhar-Nordenkampf has described the interaction of these factors as they affect the conditions necessary for optimal photosynthesis. Figures 1 and 2 show that the greatest light efficiency for optimal CO_2 fixation (CO_2 fixed per given amount of light) occurs at low light intensities, high CO_2 tensions and temperatures near 35°C ; but at atmospheric CO_2 tension (150–400 p.p.m.) the optimum temperature is near 25°C (Fig. 2). However, photorespiration is optimal at high light intensities, high temperatures and low CO_2 tensions. The amount of CO_2 (p.p.m.) in the atmosphere that can be used by the leaf to produce

1 mg CO_2 fixed per g dry weight per h (p.p.m. mg^{-1} in Figs 1 and 2) is optimal between 100 and 650 p.p.m. CO_2 at all light intensities (Fig. 1) and between 150 and 1,000 p.p.m. CO_2 at all temperatures studied (Fig. 2). Similarly CO_2 "fertilisation", which is the proportion of added CO_2 fixed for a given amount of CO_2 in the atmosphere ($\Delta\text{mg}/\Delta\text{p.p.m.}$, Figs 1 and 2), is optimal in the range between 150 and 400 p.p.m. CO_2 at high light intensities (Fig. 1) and high temperatures (Fig. 2). Figure 3 depicts the points of optimal use of light intensity and CO_2 tension for maximal photosynthesis. From Fig. 3 it may be noted that below light intensities of $0.02 \text{ cal cm}^{-2} \text{ min}^{-1}$ and 150 p.p.m. CO_2 concentration, there is no net photosynthesis. This implies that an increase in net photosynthesis can only be achieved by increasing both these factors above the minimal levels. It is also apparent from Fig. 3 that at any given temperature (15°C for example) any variation in either atmospheric CO_2 or light intensity (within the hatched area) will result in the same net photosynthesis. For example at 25°C at light intensities of $0.15 \text{ cal cm}^{-2} \text{ min}^{-1}$, varying the CO_2 concentration between 250 and 1,000 p.p.m. has no effect on net photosynthesis. Similarly at 300 p.p.m. CO_2 and at 25°C , varying the light intensity between 0.05 and $0.16 \text{ cal cm}^{-2} \text{ min}^{-1}$ has no effect on net photosynthesis. But by increasing the light intensity over the range 0.08 to $0.34 \text{ cal cm}^{-2} \text{ min}^{-1}$ and CO_2 concentration over the range 300 to 1,500 p.p.m. at 25°C it is possible to increase net photosynthesis from 25 to 100 mg CO_2 g dry weight per h. Thus only by CO_2 enrichment and high light intensities is it possible to counteract the depression of C_3 net photosynthesis that normally occurs at 35°C .

It is of practical importance that CO_2 enrichment has the added advantage of not only decreasing photorespiration but also increasing nitrogen fixation by symbiotic bacteria in root nodules. It has been suggested that the amount of photosynthate available to the nodule may be the primary factor for limiting nitrogen fixation, and any conditions that increase available photosynthate to the bacteria in the roots would increase nitrogen fixation.

High light intensities in combination

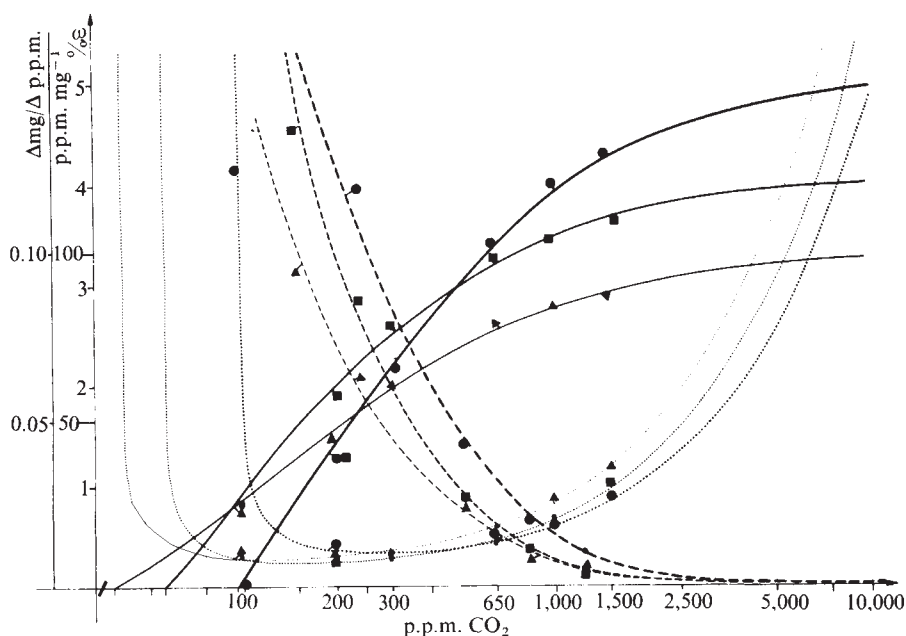


Fig. 1 Effect of CO_2 concentration at four light intensities ($\circ$, 0.556; $\bullet$, 0.265; $\blacksquare$, 0.150; $\blacktriangle$, 0.065 $\text{cal cm}^{-2} \text{ min}^{-1}$) on net photosynthesis at 25°C . —, % light efficiency, ϵ (CO_2 fixed per given amount of light); — — —, " CO_2 fertilisation" ($\Delta\text{mg}/\Delta\text{p.p.m.}$); , CO_2 in atmosphere used to fix 1 mg CO_2 per g dry weight per h (p.p.m./mg). CO_2 concentration is given in p.p.m. on a log scale.

with atmospheric O_2 pressures (high) and CO_2 tension (low), result in the production of oxygen free radicals during the normal photosynthetic electron transport processes. Bolhar-Nordenkamp suggests that photorespiration occurs as a result of the overproduction of oxygen radicals and the function of photorespiration is thus to prevent any photo-oxidation reactions which could otherwise easily damage the cell. This is thought to be by way of an interaction with glycolic acid metabolism. The mechanism of photorespiration is still controversial, but it is believed that the metabolism of glycolate which may be derived from one or more intermediates of the C_3 pathway, is intimately involved.

Several reactions have been proposed to account for the CO_2 release from glycolate. It is generally accepted that glycolate is oxidised to glyoxylate by glycolate oxidase in the peroxisomes; the glyoxylate is then transaminated to glycine. Glycine is transported out of the peroxisomes into mitochondria where it is converted to serine and CO_2 ; this step is possibly associated with some degree of energy conservation. Recent results in fact show that intact spinach leaf mitochondria can oxidise glycine with good respiratory control and its oxidation is coupled to three phosphorylation sites. The conversion of glycine to serine is currently considered to be the major reaction releasing CO_2 during photorespiration at temperatures up to $25^\circ C$. An alternative reaction leading to the evolution of photorespiratory CO_2 is the direct decarboxylation of glyoxylate to formate, in both peroxisomes and chloroplasts, by a non-enzymatic reaction with H_2O_2 . It is thought unlikely, however,

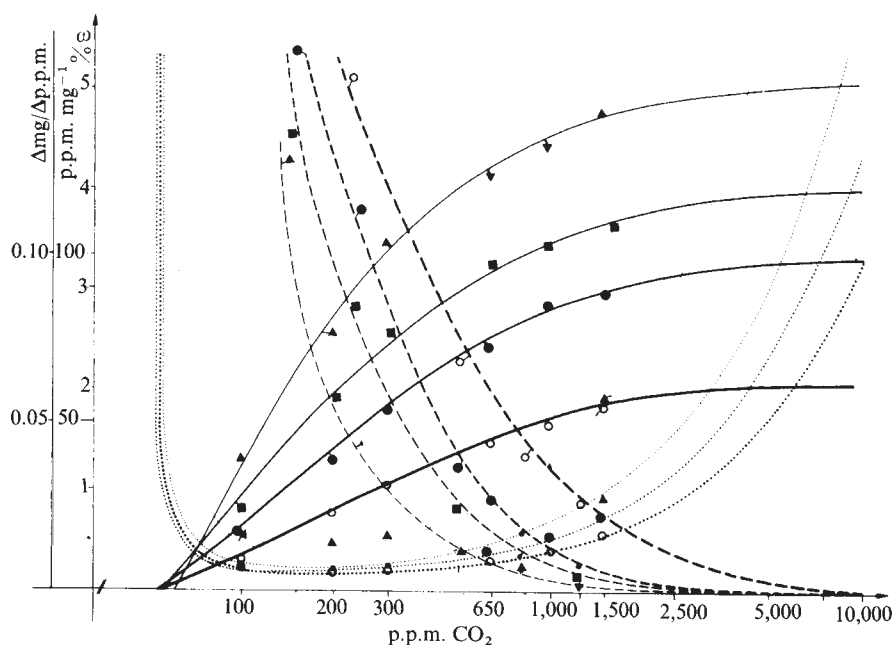


Fig. 2 The effect of CO_2 concentration at three temperatures (Δ , $15^\circ C$; $\blacksquare$, $25^\circ C$; $\bullet$, $35^\circ C$) on net photosynthesis at a fixed light intensity ($0.15 \text{ cal cm}^{-2} \text{ min}^{-1}$). Other symbols as in Fig. 1.

that this non-enzymatic decarboxylation in peroxisomes contributes more than 10–20% of the CO_2 released during photorespiration (see Halliwell *New Phytol.* 73, 1075; 1974).

The superoxide free radical anion (O_2^-) (produced by the reduction of O_2 in a one-electron step) may also play an important part in photorespiration. Although there is sufficient catalase present in the peroxisomes to destroy 80% or more of the H_2O_2 generated during glycolate oxidation, mitochondrial fractions from spinach leaves and purified

mitochondria from some etiolated tissues have been shown to generate H_2O_2 *in vitro*. The production of H_2O_2 by isolated chloroplasts is a well known phenomenon. Superoxide dismutases which catalyse the breakdown of O_2^- to O_2 and H_2O_2 have been found in the chloroplasts and mitochondria of spinach leaves. Since both mitochondria and chloroplasts contain systems which can generate O_2^- , it is likely that superoxide dismutases function to remove it. Their significance in relation to CO_2 release in photorespiration is, however, still to be evaluated. □

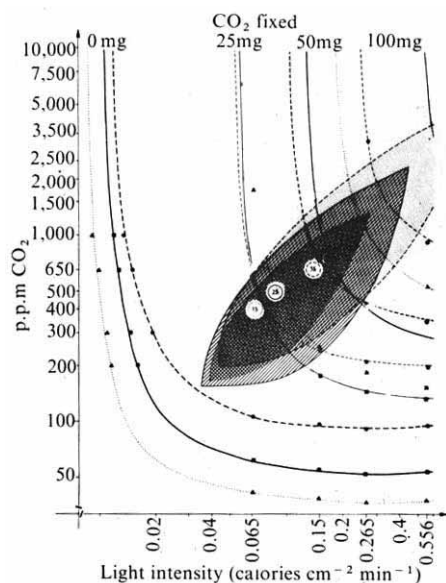


Fig. 3 Points of equal net photosynthesis at three temperatures and variable CO_2 concentration and light intensity. The figure depicts the points of optimal use of light intensity and CO_2 tension for maximal photosynthesis. Symbols as in Fig. 2.

Growth hormones and serum factors

from Robert Shields

It has been apparent for some time that only around 10% of the insulin-like activity in serum can be suppressed by insulin antibodies, but only now has the nature of the rest of the activity become clearer. Recent reports show that this non-suppressible insulin-like activity (NSILA) is due to a peptide structurally related to insulin, and that this peptide is probably the major growth hormone in the body as well as being a "serum factor" for *in vitro* cell culture.

Attempts to purify NSILA have been hampered by the fact that no organ seems to have high levels of

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activity and the concentration of the protein in plasma is very low. Industrial scale procedures are needed to isolate only a few milligrams of material from several tons of human plasma. The usual purification procedure begins with the fractionation of plasma by the Cohn procedure (essentially a series of controlled ethanol precipitations under increasingly acid conditions). Acid/ethanol precipitation of the Cohn IV precipitate (weighing a few kg) produced a small amount of soluble activity (NSILA-S) with most of the activity remaining in the precipitate (NSILA-P). It was thought therefore that this represented two distinct molecular species of molecular

weights 6,000 and 90,000 respectively. But it now seems likely that NSILA-P is an artefact generated by the acid/ethanol procedure¹, since with acid extraction alone 90% of the activity is recoverable in the soluble fraction².

Using the insulin-like effects of the material on adipose tissue as a guide, further purification was achieved by molecular sieve chromatography, preparative gel electrophoresis and ion exchange chromatography; finally the activity was isolated in two structurally similar peptides². Since the plasma used as starting material for the isolation of NSILA was pooled from several thousand individuals it is not clear whether these two peptides are the products of two genetic loci in an individual or are allelic variants in the population. Preliminary sequences of the N-terminal region of NSILA-I and NSILA-II reveal that the proteins are very similar to each other and that 50% of the first 30 residues of NSILA-I are identical to the 30 residues of the insulin B chain. This has led Rinderknecht and Humbel to propose that proinsulin may have evolved through gene duplication of an ancestral gene coding for a molecule very similar to NSILA³. It will be interesting to see if the remaining 20 residues of NSILA bear any resemblance to the C peptide of proinsulin which connects the N terminal B chain to the insulin A chain.

Role for NSILA

Many cells examined seemed to have membrane receptors for both insulin and NSILA^{4,5}. The sites are distinct but not surprisingly, in view of the homology of NSILA and insulin, high doses of one hormone will interact with the receptor for the other. This explains why high concentrations of insulin may mimic the effects of physiological levels of NSILA and *vice versa*^{6,7}. The physiological role of insulin is relatively well-understood, what then is the role of NSILA? It is obviously not active *in vivo* as an insulin analogue since diabetic animals have normal plasma levels of NSILA¹. It turns out that NSILA is probably

the major mediator of the effects of the classic trophic hormone, growth hormone.

Growth hormone and somatomedin

The role of growth hormone was determined historically by the observation that hypophysectomised animals fail to grow normally and that this defect can be largely repaired by injection of growth hormone (GH). But attempts to demonstrate the action of GH on skeletal tissue *in vitro* failed until it was realised that GH elicited the appearance of a second hormone that was responsible for many of its attributed physiological effects. This second hormone was originally termed sulphation factor since it was active in promoting sulphate uptake into cartilage. It is now clear however, that this is only one of its activities and amongst other activities it also possesses non-suppressible insulin-like activity. The factor was renamed somatomedin to imply that it mediated the effects of GH (somatotrophin)⁸.

Attempts to purify somatomedin from plasma proceeded on similar lines to those for NSILA, and the purification of a number of peptides with somatomedin (SM) activity has been reported. The biological assay used to guide purification was the incorporation of radioactive sulphate into cartilage, probably a very indirect assay for protein synthesis in the chondrocyte. Indeed, somatomedins have been shown to stimulate RNA and protein synthesis as well as other components of the pleiotypic response in skeletal tissue⁹. Two sources of cartilage are used to follow the purification of somatomedin activity, rat costal cartilage⁹ and chick pelvic leaflets¹⁰. Using the chick assay as a guide the isolation of two somatomedins has been described¹¹. Somatomedin A (SMA) is reported to be a neutral peptide active in the chick assay and somatomedin B (SMB) is an acidic peptide with no activity in the chick cartilage assay but is a growth factor for cultured cells¹². Using the rather more tedious and expensive rat cartilage assay another group of

workers have isolated a basic protein which they term somatomedin C (SMC)⁹. There is now an overwhelming body of evidence suggesting that SMC, SMA and NSILA purified by different groups are either very closely related, or most probably variants of the same molecule, whose active form in the plasma produces the effects of GH.

The probable relationship of SMA and NSILA is suggested by their similar purification schedules, their closely similar physicochemical properties and the fact that at no stage of their purification could insulin-like activity be dissociated from somatomedin activity¹³. Furthermore, these proteins bind to receptors with similar affinity and are displaced by high doses of insulin, and both activities are growth hormone dependent in that levels are low in pituitary dwarfs and high in acromegalics. Initially there was some doubt about the relationship of SMA to SMC and NSILA, as SMC and NSILA were only weakly active in the chick cartilage assay used to detect SMA¹⁴. However, the purified SMA peptide was itself hardly active in this assay although it was active in the rat cartilage system and so possessed SMC activity¹⁵. This puzzle was resolved when it was demonstrated that purified NSILA required the presence of another unidentified serum factor (not under GH control) in order to show sulphation factor activity in the chick assay¹⁶, although stimulation by NSILA of the pleiotypic response in chick cartilage was independent of this serum factor. Furthermore, SMA and SMC compete for the same receptor¹³. Definitive proof that SMA, SMC and NSILA are related must await comparative amino acid sequence data but the need for large amounts of pure material may mean that this proof may take some time.

Trophic action of NSILA

The trophic action of NSILA is not confined to chondrocytes; the factor is very active on cultured animal cells in promoting the parameters of the pleiotypic response and DNA synthesis^{2,17}. Insulin at pharmacological levels has often been used as a growth factor for cultured cells, and the reason for its effect can now be seen to be due to its cross reaction with NSILA receptors on the cell surface. In fact, physiological doses of NSILA have marked trophic effects in cell culture, so NSILA is probably an important member of the elusive serum factors and is quite possibly the one of major physiological relevance.

With so much NSILA in the plasma why aren't animals permanently hypoglycaemic? The answer seems to be that plasma contains a specific binding protein for NSILA which limits its

Table 1 Some of the properties of NSILA and somatomedin

Property	NSILA	SMA	SMB	SMC	Insulin (High doses)
Dependent on GH concentration	+	+	+	+	—
Carrier protein present in serum	+	+	—	+	—
Insulin-like activity in fat cells	+	+	—	+	±
Sulphation factor activity in chick cartilage*	±	±	—	—	±
Sulphation factor activity in rat cartilage	+	+	—	+	+
Growth promoting activity in cells <i>in vitro</i>	+	—	+	+	+
Competition with high doses of insulin for receptor	+	+	—	+	+

*Full activity in this assay probably requires the presence of another serum factor.

diffusion across capillaries into the extravascular space¹⁸. This helps to explain why the concentration of NSILA in lymph is half that in plasma¹⁹ whereas insulin (which has no binding protein) can equilibrate between these two compartments. The most intriguing feature of this binding protein is that its concentration in the plasma is regulated by GH^{20,21} and the binding of NSILA to its binding protein prolongs NSILA's half-life in plasma from a few minutes to several hours. The release of NSILA from its binding protein is thus a major factor in governing the availability of this growth promoting hormone to the tissues of the body. This also explains why searches in various organs for high concentrations of NSILA were unsuccessful; NSILA is concentrated in one of the largest 'organs' of the body—the plasma.

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- ² Rinderknecht, E. & Humbel, R. E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2365 (1976).
- ³ Rinderknecht, E. & Humbel, R. E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4379 (1976).
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- ⁸ Daughaday, W. H. *et al.* *Nature* **235**, 107 (1972).
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- ¹⁰ Hall, K. *Acta endocr.* **63**, 338 (1970).
- ¹¹ Uthne, K. *Acta endocr. Suppl.* **175**, 1 (1973).
- ¹² Westermarck, B. *et al.* *Expl. Cell Res.* **96**, 58 (1975).
- ¹³ Hall, K. *et al.* in *Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 19 (Academic, New York, 1975).
- ¹⁴ Sievertsson, H. *et al.* in *Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 47 (Academic, New York, 1975).
- ¹⁵ Van Wyk, J. *et al.* in *Advances in Metabolic Disorders* (eds Luft & Hall) **8**, 127 (Academic, New York, 1975).
- ¹⁶ Froesch, E. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2904 (1976).
- ¹⁷ Morrell, B. & Froesch, E. R. *Eur. J. clin. Invest.* **3**, 119 (1973).
- ¹⁸ Zapf, J. *et al.* *Arch. Biochem. Biophys.* **168**, 638 (1975).
- ¹⁹ Cohen, K. L. & Nissley, J. P. *Endocrinology* **97**, 654 (1975).
- ²⁰ Cohen, K. L. & Nissley, J. P. *Acta endocr.* **83**, 243 (1976).
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- ²² Znigg, A. E. & Froesch, E. R. *Diabetologia* **9**, 472 (1973).
- ²³ Takano, K. *et al.* *Acta endocr.* **80**, 14 (1975).
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Toxin subunits

from a Correspondent

DURING the past couple of years knowledge of the mechanism of action of bacterial toxins, particularly cholera, tetanus and diphtheria toxins, and studies of hormone action have pointed out several areas of similarity in these diverse biological systems. Apparently, similar surface membrane receptors may be part of the systems responsible for uptake of some toxins and hormones, an obligatory step in the biological action of these substances on cells. Perhaps the most striking result comes from L. D. Kohn and his

collaborators who find some homologies in peptide sequences between the cholera toxin and thyroid-stimulating hormone (TSH). Mullin *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 842, 1679; 1976; Ledley *et al.* *Biochem. biophys. Res. Commun.* **69**, 852; 1976; Meldolesi *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4060; 1976). For this reason alone, current research on bacterial toxin structure and action has wide implications.

Several recent papers provide detailed information on the structure of tetanus and diphtheria toxins. These two toxins superficially have very similar structures and in the native state consist of single polypeptide chains which are easily 'nicked' by proteases into two polypeptides held together by disulphide bonds. Both polypeptide 'subunits' are essential for the activity of either toxin on intact cells. One of the fragments of 'nicked' diphtheria toxin (A subunit), however, is active in inhibiting protein synthesis in cell-free extracts by inactivating elongation factor 2 (translocase) in precisely the same way as intact toxin exerts its killing effect on unbroken cells. The second polypeptide subunit of nicked diphtheria or tetanus toxin then is reasonably assumed to be required to attach the toxin to the surface membrane of whole cells. Until recently this point was hard to establish for diphtheria toxin, since the B subunit is insoluble under normal conditions after disulphide bond cleavage of nicked toxin and may be kept in soluble form only in urea or other protein denaturants. Two groups have now devised methods for the isolation of stable B fragments. Helting and Zwisler (*Behring Inst. Mitt.* No. 59, 92–101, 1976) treated diphtheria toxin with formaldehyde under mild conditions before trypsinisation and reduction of disulphide bonds. The B fragment was immunogenic in rabbits or guinea pigs and the specific antibodies elicited by immunisation blocked the lethal action of diphtheria toxin in animals or whole cells, presumably by interfering with the interaction of the appropriate peptide sequence of the toxin with specific surface membrane receptors. In contrast, these antibodies failed completely to affect the enzymatic activity of isolated fragment A in inhibiting *in vitro* protein synthesis. More direct evidence for fragment B interactions with cells was obtained by Everse *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **74**, 472; 1976). The chains were stabilised either by using low concentrations of sodium dodecyl sulphate or by sulphonation. Solutions containing B fragment protected HeLa cells from the effect of intact diphtheria toxin, while A fragment was completely inactive. Although rather large

amounts of B fragment were required for protection against the toxin the effect does seem to be specific and strengthens the idea that the B fragment of the toxin molecule probably exerts the initial interaction with cell membrane receptors. Everse *et al.* also showed that 'unnicked' toxin was inactive when tested against HeLa cells under conditions where the 'nicked' toxin was highly cytotoxic. Probably, therefore, interaction of the toxin with cell surface receptors takes place most efficiently after 'nicking', a point that is interesting in the context of reports that some tumour cells are more sensitive to the action of diphtheria toxin than normal cells (Iglewski & Rittenberg *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2707; 1974; Pappenheimer & Randall *Proc. natn. Acad. Sci. U.S.A.* **72**, 3149; 1975) and the induction of proteolytic enzymes in some transformed cell lines (see for example Chen & Buchanan *Proc. natn. Acad. Sci. U.S.A.* **72**, 1132; 1975).

The subunit of 'nicked' tetanus toxin analogous to fragment B of diphtheria toxin has also been identified recently (Helting & Zwisler *J. biol. Chem.* **252**, 187; 1977; Helting *et al.* *J. biol. Chem.* **252**, 194; 1977). The larger of the two polypeptides (heavy chain) derived after mild trypsinisation retained all of the receptor binding activity of the native toxin and a specific antiserum directed against a part of the heavy chain completely prevented activity of whole toxin. These new techniques, therefore, make available stable fragments of both diphtheria and tetanus toxin that are efficient in eliciting antibodies which interfere with the interactions with brain receptors and neutralise toxic action. Such vaccines may have advantages over the standard preparations against inactivated toxoids. Reactions of delayed hypersensitivity are known in immunisation, particularly of older children, for example (see Helting & Zwisler *op. cit.* 1976) and the use of peptide fragments lacking the antigenic determinants producing these undesirable responses would be a distinct advance. □

Microtubes

from Peter J. Smith

EVERY now and then someone decides to investigate a terrestrial phenomenon which has been known for many decades, if not a hundred years or more, but which has never before been studied in any detail. Quite why certain phenomena should be ignored for so long is far from clear. Some have probably been considered trivial—of interest in themselves, perhaps,

but with no potential for influencing other branches of the Earth sciences and thus of no significance to the geological mainstream. But not all examples of scientific neglect can be explained away so easily. Consider, for instance, the microscopic tubes, or microtubes, in igneous rocks. There must be a reason for their presence; and an obvious case can be made for saying that they should be able to provide valuable information on the history of their hosts. Yet unlike primary and secondary inclusions in rocks, about which there is now a vast literature, they have been almost completely overlooked.

Richter and Simmons (*Earth planet. Sci. Lett.* **34**, 1; 1977), who have begun a detailed study of microtubes, define them as cavities of circular or elliptical cross section and having diameters generally less than $5\text{ }\mu\text{m}$ and lengths greater than $10\text{ }\mu\text{m}$. They may be hollow or partly or completely filled with a solid phase, but they are distinct from the better-known euhedral needle-like inclusions of minerals such as rutile and apatite. They are also distinct from microcracks, which have one dimension (generally less than a few micrometres) much smaller than the other two (greater than several tens of micrometres). They do not have faceted walls and so can be neither negative crystal forms of the host grain nor the result of acicular inclusions. Yet they are evidently single crystal phenomena, for they never cross grain boundaries.

The first known reference to microtubes was by Sorby (*Q. Jl Geol. Soc.* **14**, 453; 1858) who recognised several isolated examples in vein quartz but made little comment about them. Then Rosenbusch (*Mikroskopische Physiographie der Massigen Gesteine*, Stuttgart, 1907) found very fine cylindrical canals in samples of deformed granite, concluding that they were formed by stresses incurred during mountain building; and Honess (*The Nature, Origin and Interpretation of Etch Figures on Crystals*, New York, 1927) later discovered tubular etch pits in natural quartz. More recently only a handful of workers have mentioned the presence of microtubes and even fewer have commented on them more than briefly, thereby giving the impression that such features are rare. Yet they are very far from rare. They may have been overlooked by many petrographers; but Richter and Simmons have observed them in granite, quartz monzonite, syenite, granodiorite, anorthosite, diabase, gabbro and even lunar basalt, the minerals involved being quartz, K-feldspar,

plagioclase, muscovite, biotite, pyroxene and olivine.

So microtubes are evidently more common than previously supposed. But how do they form? Richter and Simmons conclude from petrographic and scanning electron microscope studies that there is no single process of formation but at least three. It is clear from their groupings, associations and spatial relationships that many microtubes arise from the partial annealing of microcracks. Others, however, appear to have resulted from the etching of crystal defects such as dislocations by naturally occurring fluids, whilst still others are evidently primary features produced by the inclusion of fluid material during crystal growth.

There may be other, as yet undiscovered, ways in which microtubes

form. But such diversity is an advantage, for it suggests even more strongly that the study of microtubes may throw light on the various physical and chemical conditions to which many igneous rocks may be subject both during and subsequent to their formation. Microtubes resulting from annealing might give information on a rock's deformation history, etch tubes could possibly provide evidence of the types of fluid that have circulated through a rock; and primary tubes should provide clues to the conditions obtaining during crystal growth. For the time being Richter and Simmons pursue these possibilities no further; but it is clear from the progress they have made so far that it may have been a mistake to have given microtubes so little attention in the past.

Sex ratios in social insects

from John Krebs

In a paper published just over a year ago, R. L. Trivers and H. Hare (*Science* **191**, 249; 1976; see also Krebs & May *Nature* **260**, 9; 1976) reported results which in one fell swoop appeared to verify Fisher's theory of the sex ratio, Hamilton's kinship theory of altruism in social hymenoptera, and Trivers's own ideas on manipulation of parents by offspring; a virtuoso display indeed! This seminal and apparently incontrovertible piece of work has now come under vigorous attack from R. D. Alexander and P. W. Sherman (*Science* **196**, 494; 1977).

To recap on the previous story, Trivers and Hare accounted for the apparent altruism of sterile female workers of social hymenoptera such as bees and ants in the following way. As a result of the sex determination system, in which haploid males develop from unfertilised eggs, the diploid females share $3/4$ of their genes by direct descent with their full sisters, and $1/4$ with full brothers. The diploid mothers share half their genes with sons and daughters alike. As W. D. Hamilton first showed, this state of affairs means that a female who has the "choice" between producing her own daughters or rearing reproductive younger sisters makes a genetic profit by doing the latter. However, in order to explain fully the evolution of sterile worker castes in terms of Hamilton's kin selection theory, one has to consider why the workers also rear brothers (with whom they share only $1/4$ of their genes). Trivers and Hare, following up a suggestion of Hamilton, pointed out that workers should be

more prone to rear reproductive sisters than drones. More precisely, they should invest three times as much effort in rearing new queens. If drones are only a third as common as queens they will have on average three times the mating success, which as far as a worker is concerned, exactly compensates for the threefold difference in relatedness. In any typical diploid outbreeding species, the mother's optimal ratio of investment in daughters and sons is 50:50. (This is the only stable equilibrium since the rarer sex is always at an advantage.) Trivers's and Hare's striking finding was that the ratio of investment in 21 species of monogynous (one active queen per colony) ants was close to 3:1 in favour of females, just as predicted by the simplest genetic model based on the assumption of worker success in manipulating the sex ratio to their advantage. As a 'control', Trivers and Hare showed that the investment ratio was much closer to 1:1 in slave-making ants and non-social hymenoptera where the queen presumably achieves her optimum. Far from the picture of selfless worker altruism, these remarkable results suggest that the queen is, in effect, farmed by the workers as a reproduction machine.

Alexander and Sherman question not only the factual basis and statistical treatment of Trivers's and Hare's evidence but also point to an alternative interpretation. The critique is detailed and complex and I can only highlight a few points. Three main

challenges to the factual evidence emerge: multiple matings are commoner than Trivers and Hare assume; workers often lay eggs which develop into drones; and the investment ratio in slave-making ants, termites, and non-social bees and wasps seems to be slightly biased towards females.

The calculations of degree of relatedness on which Trivers's and Hare's predictions are based depend critically on their assumption that the workers and the reproductive females these rear are full sisters. If the queen mated with three males and mixed the sperm at random, the whole argument for a bias on the part of workers would collapse. Alexander and Sherman point out that multiple matings are quite common in social hymenoptera. But the problem is that single matings are virtually impossible to prove even if they are common. One might also add that the mating habits of present-day queens are probably less critical to Trivers's and Hare's argument than those of queens during the early evolution of eusociality: studies of mating in the semisocial hymenoptera suggest that monogamy may have been quite common at this intermediate evolutionary stage.

The kinship theory of social hymenoptera does in fact predict that workers should attempt to lay eggs, since by so doing they substitute sons (degree of relatedness $1/2$) for brothers (to whom they are related by only $1/4$). This point was discussed at length by Trivers and Hare, who consider egg laying to be an alternative strategy to manipulating the investment ratio by workers. The only point in question is whether the monogynous ants selected for analysis by Trivers and Hare are species in which the sex ratio is influenced by laying workers, a point on which there seems to be no concrete evidence.

Alexander's and Sherman's doubts concerning the claims of Trivers and Hare that the ratio of investment in slave-making ants, termites, and non-social hymenoptera is close to $1:1$ stem from the fact that the original data are incredibly variable, some nests showing a strong female bias and others a trend towards males. Alexander and Sherman conclude that the termites show a slight female bias (kinship theory predicts no bias) and that some of the data for unsocial bees and wasps used by Trivers and Hare may be subject to the sampling bias. Nevertheless they admit that the basic trend—a stronger female bias in investment ratio in monogynous ants than in non-social hymenoptera—is still valid.

Much more important than these hair-splitting criticisms, is Alexander's and Sherman's suggestion that a plausible alternative hypothesis could

account for the results obtained by Trivers and Hare. Interestingly, this alternative idea was also developed by W. D. Hamilton, who pointed out that Fisher's theory of equilibrium sex ratios does not hold when there is inbreeding and siblings compete with each other for matings (as males usually compete for females rather than *vice versa*, the argument can be taken to refer to competition between brothers). Suppose, for example, that a female's daughters are all fertilised by their brothers. The mother's best strategy is to produce just enough males to ensure that all daughters are fertilised, putting most of her resources into female offspring. Fisher's $1:1$ equilibrium no longer holds, because the expected reproductive success of sons does not depend on what goes on in the rest of the population. Whenever this sort of competition between brothers occurs the optimal sex ratio will be biased towards females, the exact bias depending on the degree of inbreeding.

If, as Alexander and Sherman suggest, inbreeding and sibling competition for mates is common in social hymenoptera, the biased investment ratio might be optimal for the queen and not, as Trivers and Hare suggest, for the workers, who would like a still greater bias in favour of females. The two hypotheses, both apparently consistent with the data, are not necessarily mutually exclusive. The mean investment ratio for the monogynous ants analysed by Trivers and Hare was about 3.5, slightly in excess of the predicted 3.0. This could conceivably be an effect of both mating competition and worker manipulation, although one cannot check this as Alexander and Sherman are unable to predict an optimal investment ratio for the queen without more information on the extent of mating competition.

Trivers's and Hare's hypothesis, on the other hand, not only predicts with reasonable success (given the difficulty of measuring 'investment') the observed bias in monogynous ants, but also predicts the difference in investment ratio between monogynous ants on the one hand, and the slave-making ants, non-social bees and termites on the other. In order to account for this difference, Alexander and Sherman have to invoke the (plausible) argument that inbreeding is less common in the second group than in the first. (They also point out that among the non-social hymenoptera, a slight female bias in the sex ratio is more often observed in those species which do have inbreeding).

To sum up, although the evidence is not as clear cut as it first appeared, Trivers's and Hare's hypothesis still provides the more parsimonious pre-

dictive explanation of the data, and what is more, it also predicts other observations, for instance laying by workers, and the absence of sterile males in the hymenoptera but not in the termites. Nevertheless the sex ratio is almost certainly modified to some extent by inbreeding. □

Low temperature electron microscopy

from Patrick Echlin

The 1st International Meeting on Low Temperature Biological Microscopy was held in Cambridge 4–7 April, 1977 under the auspices of the Royal Microscopical Society. Edited versions of all the papers will appear later this year in the *Journal of Microscopy*.

ALTHOUGH low temperature techniques are now assuming a greater significance in biological investigations, many biologists are unhappily unaware of the complex physicochemical properties of ice and the nature of the freezing process in cells and tissues. This interdisciplinary meeting sought to redress this situation by bringing together ice physicists, physical chemists, cryobiologists, engineers and microscopists to see whether these techniques are indeed fulfilling the promise of perfect preservation of ultrastructure and functional physiology. All the participants overtly or tacitly accepted the dictum that 'cold is good' but there was extensive discussion as to what constitutes 'good' in relation to ultrastructure, analysis and vitality. J. Farrant (Clinical Research Centre, Harrow) showed that with a few exceptions good ultrastructure, retention of soluble constituents and preservation of vitality were probably always likely to be mutually exclusive. This, the cryobiologist's trinity, could only be achieved by vitrification, a process rarely achieved in biological tissues.

Various techniques which would help overcome the problems associated with ice crystal damage were discussed. H. Sitte (University of Homburg-Saar), H. Muller (Technical High School, Zurich), W. Bald (University of Oxford) and L. Seveus (Stockholm) described new fast-freezing techniques, all of which could theoretically give sufficiently fast cooling velocities in small pieces of biological material to bring about a shift from heterogeneous nucleation to homogenous nucleation with the consequent formation of very small ice crystals. Alter-

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native approaches were offered by T. Nei (University of Sapporo, Japan), H. Skaer (University of Cambridge) and A. Walter (Clinical Research Centre, Harrow) who advocated the use of penetrating and non-penetrating cryoprotectants. Nei showed images of remarkable preservation in red blood cells frozen and thawed in the presence of 20% glycerol, and Skaer gave details of some high molecular weight polymers which although they do not enter cells, limit the size of intracellular ice crystals to nanometre dimensions even in pieces of tissue 1 mm in diameter. Such polymers have important implications in the preparation of tissues for freeze fracture and X-ray microanalytical studies which are best carried out on chemically unperturbed tissues.

There was extensive discussion on the process of sectioning and freeze fracturing and the nature and extent of the artefacts associated with these techniques. A. Saubermann (Harvard Medical School) demonstrated that the process of cutting frozen material is a mixture of sectioning and multiple fracturing depending on temperature, cutting speed and knife angle, and U. Sleytr (Vienna) and T. Robards (University of York) showed that plastic deformation can occur at temperatures well below the glass transformation point of ice. Sufficient energy is released during cleavage to deform biological material and great care must be taken when interpreting freeze fracture images. The same point was taken up by D. Branton (Harvard University) and S. Bullivant (University of Auckland) who showed that some of the artefacts associated with membrane structure are produced either during specimen pretreatment and freezing or simply by the collapse of the lipid layers after membrane cleavage.

Low temperature techniques are clearly no longer the preserve of the morphologists and a number of papers were given showing how both frozen-hydrated and frozen-dried tissues and sections could be used for the analysis of diffusible ions and electrolytes. K. Zierold (Max Planck Institute, Dortmund) and M. Sjostrom (University of Umea, Sweden) gave details of low temperature X-ray analytical studies on mammalian muscle. T. Barnard (Wenner-Gren Institute, Stockholm) related the diffusion of electrolytes in frozen sections of adipose tissue to changes in the electron density of the sections. One of the main problems of low temperature microscopy is to ensure that the biological material stays cold enough during preparation and examination. P. Echlin (University of Cambridge) considered these problems in relation to cryofractured frozen bulk samples and sections examined at

low temperatures in the scanning electron microscope and suggested a number of criteria which could be used to judge the hydration state of material in the microscope. Such problems are less exacting for the smaller samples examined by K. Taylor (MRC Laboratory of Molecular Biology, Cambridge) in the transmission microscope who used the disappearance of the ice diffraction pattern as an indicator of specimen hydration in catalase crystals and bacterial cell walls. There were close similarities between the negative stain images of catalase and the hydrated crystals, and it looked as if the ice was acting rather like a negative stain. The earlier hopes that low temperatures might dramatically diminish radiation damage in high resolution images were dashed when R. Glaeser (University of California, Berkeley) showed that depending on the specimen, the damage is only lessened by a factor of two when operating at liquid nitrogen temperatures and a factor of five when operating only a few degrees above absolute zero.

There was considerable discussion on the interpretation of morphological data obtained from frozen sections and fractures. T. Gulik-Krzywicki (CNRS Gif-sur-Yvette) showed that it is possible to correlate low temperature X-ray diffraction patterns with images obtained by freeze fracture electron microscopy, and H. Chanzy (CNRS Grenoble) described a technique for low temperature electron diffraction. Whereas these specialised techniques and the use of image processing can help with the interpretation of the inert freeze fracture images, such methods are of little use for unstained frozen sections which give images containing few of the subcellular features recognisable from conventional microscopy. Cryohistologists are going to have to re-educate themselves to recognise images in material produced and examined at low temperatures. □

Head-on proton collisions

from Peter Landshoff

MOST of the information on the structure of protons has been obtained by bombarding them with very high energy electrons, and these are the experiments that have provided strong, though indirect, evidence that protons contain quarks. The quarks apparently

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A hundred years ago

THE Superintendent's Report on the Botanic Garden and Public Plantations for 1875-76 has recently been officially published in Jamaica. It deals almost entirely with plants of economic value, foremost of which is the coffee, the ordinary kind (*Coffea arabica*), apparently giving way to its formidable rival *Coffea liberica*, which was introduced to Jamaica in 1874, and is now thriving, especially in some districts. In one situation, at a height of about 1,000 feet above the sea, a plant that had only been planted out a little over a year has already fruited. This seems to indicate that in the course of a few years the new coffee may be widely cultivated in Jamaica from plants raised from seeds ripened in the island.

From *Nature* 16, 24 May, 73; 1877.

have very small diameters, or are maybe even pointlike, although the proton as a whole has a more diffuse structure in which the quarks are embedded. The nature of the remaining constituents is not yet clear. Certainly they include quark-antiquark pairs, some of which may be bound tightly together to form mesons. Very possibly they include also the 'gluons' that bind quarks and antiquarks together in the quantum field theories that mathematical physicists are constructing in attempts to unify the various fundamental forces of nature.

The evidence that there are quarks inside the proton is indirect, because no free quarks have yet been seen. Indeed, many physicists believe that it may not be possible for quarks to be knocked out of their parent protons. (There have been various attempts to construct such theories of 'quark confinement'.) For this reason, it is desirable to verify the quark structure in other types of experiment. Beams of high-energy muons and neutrinos have also been used to probe the proton, and the information that they give, while again indirect, seems to confirm and complement that which comes from electron scattering. A quark structure in the proton seems rather sure.

Electrons, muons and neutrinos are particularly useful as probes because their own structure is especially simple and well understood. However, additional information can be obtained by making pairs of protons collide at very high energy.

The diameter of a proton is about 10^{-15} m—much greater than that of a quark, but infinitesimal compared with the width of a laboratory proton beam. So when two high energy beams are fired at each other, as in the Intersecting Storage Rings of the European laboratory at CERN, most of the protons do not collide. Those that do nearly always are in glancing collision. The dynamics of such collisions, where only the outer parts of the two protons interact, is complicated. It does not involve the quark structure in a very direct way, and the particles produced in the interaction (mostly pions) are swept along more or less in the initial directions of the two colliding beams.

Very rarely, two protons collide head-on and in this case their quark structure may be expected to be involved rather directly. Also, the head-on collisions are quite likely to produce particles that emerge with a fast sideways motion relative to the initial beams. Although these 'large-transverse-momentum' events are comparatively very rare, they can readily be identified, and their study has been an important part of the CERN Intersecting Storage Rings research programme during the past four or five years. Some important information has come also from experiments at Fermilab in the United States.

High-energy proton-proton collisions produce a large number of additional particles, making the analysis of the interesting events complicated. So far the analysis cannot be complete because of the limitations of the available particle detectors. However, several of the CERN experiments have recently observed an interesting "jet structure" in the large-transverse-momentum events: the sideways-moving particles emerge together in more or less the same direction, so as to form a jet of particles. This jet structure has been seen most clearly in three experiments using the CERN Split Field Magnet facility. These experiments were performed by a group from CERN, by a CERN-Collège de France-Heidelberg-Karlsruhe collaboration, and by a British-Scandinavian-French collaboration. More recently, a collaboration from Caltech, UCLA, Fermilab, Chicago and Indiana has reported results from a jet study at Fermilab. The total momentum of the two protons that collide is zero, so there are two jets, one emerging to each side of the initial beam direction (see Fig. 1). In addition to the two jets, several particles are produced that have small transverse momentum, as in the ordinary, glancing collisions.

Pairs of jets are known also to be a feature of collisions of high-energy electrons with positrons. In these col-

Transcriptional control of fertility

from J. R. Saunders

A RECENT paper from Neil Willetts (*J. molec. Biol.* **112**, 141; 1977) provides the first biochemical evidence that the fertility of bacterial plasmids is regulated at the level of transcription. Most of the genes specifying the ability to conjugate in *Escherichia coli* comprise a single 'transfer operon' which is present on the F factor and F-like plasmids. This operon consists of genes necessary for the production of sex pili, for surface exclusion and for the metabolism of DNA during transfer. It is transcribed in the order *traA*, *traL*, *traE*, *traK*, *traC*, *traF*, *traH*, *traG*, *traS*, *traD* (Helmuth & Achtman *Nature* **257**, 652; 1975). A further gene *traJ*, has a positive controlling function over the entire operon but lies outside it. In turn the expression of *traJ* is itself subject to negative control imposed by two further plasmid genes *finO* and *finP* acting in concert. F-like plasmids isolated from the wild generally transfer at a relatively low frequency due to this control. F itself is atypical in that it is a naturally occurring *finO*⁻ mutant and consequently transfers itself at a high rate. However F will recognise the *finO* product specified by other F-like plasmids and if present in the same cell such plasmids inhibit F fertility producing the *Fin*⁺ (*fin*⁺) phenotype.

Genetic tests have established that the gene products of the transfer operon are absent during *FinOP* fertility inhibition. Hitherto it was unclear whether the *traJ* product is necessary for transcription or translation of the operon. Willetts has distinguished between these possibilities by first constructing bacteriophage λ transducing phages carrying either *traJ* or the *traL*, *traE*, *traK* region. He then used DNA from these phages as probes for the synthesis of specific messenger RNA during *FinOP* transfer inhibition. His hybridisation experiments indicate that whilst mRNA homologous with this '*tra*' DNA is present in donor strains carrying F alone it cannot be found during transfer inhibition of F by the F-like R plasmid R100. This suggests that the transcription of *traJ* is repressed under the latter conditions. In the absence of the *traJ* product, transcription of the transfer operon itself is either not initiated or possibly terminated prematurely shortly after initiation. It should prove possible now to extend these studies on the expression of sex factors by utilising λ *tra* phages carrying further transfer regions of F and other plasmids.

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lisions, the electron and the positron annihilate each other. There is convincing evidence that the energy that they leave behind subsequently materialises as a quark and an anti-quark, which apparently do not escape as free particles. Exactly what happens is not understood, but first they "dress" themselves somehow by interacting with each other, so that they then emerge as two jets of ordinary particles. There are also indications that similar jets result from the collisions of high-energy electrons, muons or neutrinos on protons.

Because there is good evidence that

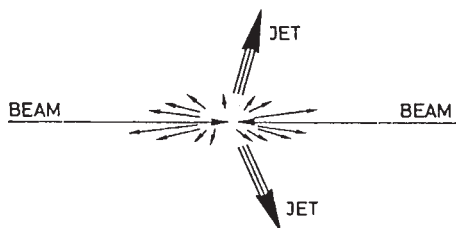


Fig. 1 The two jets produced in large-transverse-momentum collisions together with particles that have small transverse momentum relative to the incoming beams.

these jets originate from quarks, a natural guess is that the same may be true of the jets produced in the large-transverse-momentum proton-proton collisions. In the simplest model for this reaction, a quark from one of the protons scatters a quark from the other through a wide angle. The two quarks then somehow dress themselves, and result in the two jets. The remaining constituents of the two protons are responsible for the production of the longitudinally-moving particles.

There are other models that differ in detail. The experimental and theoretical question of whether the jets seen in proton-proton collisions do have the same character as those in electron-positron annihilation has yet to be answered. But it is already apparent that the large-transverse-momentum proton-proton reactions have fairly simple features which, in a way whose details are still to be determined, do reflect the quark structure of the proton. When the dynamics of these reactions are better understood, they will provide a unique way of investigating how quarks scatter on each other at high energies.

articles

Magma mixing: a mechanism for triggering acid explosive eruptions

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Injection of basic magma into acid magma causes superheating of the acid magma and vigorous convection. Vesiculation induced by convection and increased magma pressure fractures the volcanic edifice triggering an explosive acid eruption. The 1875 plinian eruption of Askja, Iceland is an example of an explosive eruption triggered by magma mixing.

THERE are many possible mechanisms of triggering explosive volcanic eruptions of acid magma. Most of these involve factors which ultimately lead to supersaturation of the melt with respect to volatiles¹. We have, however, been impressed by a number of examples of plinian pumice fall deposits where there is evidence that basic magma was intruded into acid magma and the two magmas were physically mixed by vigorous convection shortly before eruption. We will now describe this evidence and demonstrate theoretically that a number of effects of mixing are highly conducive to triggering an eruption. We propose that magma mixing is a common mechanism of triggering explosive eruptions of acid magma, although we emphasise that not all magmas that mix inevitably erupt, nor are all explosive eruptions caused by mixing.

Geological evidence

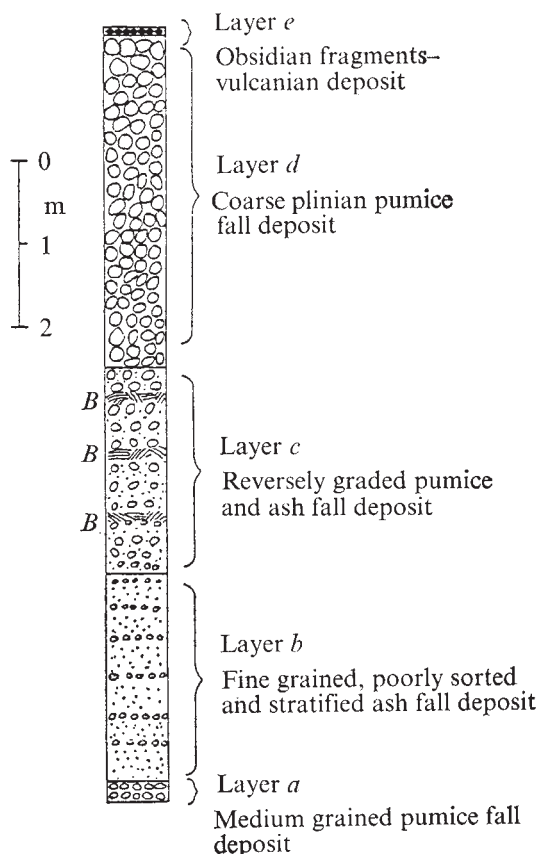
Tephra containing an intimate mixture of two or more contrasted compositions of magma is a common feature of acidic pyroclastic deposits and in our view the few examples described in the literature do not adequately reflect the abundance of mixed tephra occurrences²⁻⁶. In the cases observed by us the more basic component occurs either as bands and streaks or as irregular, rounded clots and inclusions in the acid pumice. The basic component is usually a volumetrically minor component, but shows evidence of its molten condition at the time of eruption by the occurrences of vesicles, flowage structures and its glassy condition. We now describe detailed case histories of explosive ejecta which shows these features from Askja, Iceland and Santorini, Greece.

Fig. 1 shows the stratigraphy and lithological characteristics of the mixed ejecta of the 1875 explosive eruption in Askja, Iceland. The layers formed over a period of 8.5 hours on the night of the 28–29 March 1875 (ref. 7). The plinian deposit (layer *d*) formed in six of those hours and represents 80% of the ejecta by volume. All the tephra layers show evidence of mixing and simultaneous eruption of basalt and rhyolite magma.

We have analysed the two end-member glasses which are found intimately associated in the 1875 ejecta (Table 1; analyses *a* and *b*). The basaltic component is a dark brown glass, poor in

crystal, and is similar in composition to the tholeiites which characterise the Northeastern Rift Zone of Iceland⁸. The basaltic glass occurs as irregular inclusions and streaks in rhyolitic obsidian and pumice and occasionally in bombs. More often it is found in separated, rounded, mixed dark scoria fragments. During most of the eruption the scoria clasts were finer than 1.0 cm and hence are progressively enriched in the tephra away from the source where most of the tephra less than 1 cm was deposited. Layer *c* (Fig. 1) grades laterally into a densely welded air fall tuff where both the

Fig. 1 Schematic section of the 1875 Askja tephra showing the principal lithological characteristics of the five layers. The thicknesses are the maximum observed for each layer on the north-eastern and eastern rims of Oskjuvatn. *B* refers to base surge horizons.



rhyolitic pumice and basic clasts have been deformed into fiamme, demonstrating the molten condition of both magmas. During the closing stages of eruption the size of mixed ejecta markedly increased. Large spatter bombs and mixed bombs up to 5 m diameter containing both magmas were ejected.

The rhyolitic component contains less than 1% plagioclase and clinopyroxene crystals and is relatively low in total alkalis and high in CaO and MgO (Table 1) compared to other Icelandic rhyolites⁹. Some plagioclase phenocrysts are rounded but they contain glass inclusions of rhyolitic composition showing that they formed in equilibrium with the acid liquid. The rounding could be interpreted as resorption due to superheating of the rhyolite. Rounded grey clots (0.1–1.0 cm) are found as inclusions in the rhyolitic pumice in all the layers. These inclusions contain between 40% and 50% of plagioclase and pyroxene microphenocrysts set in a pale brown glass. The detailed petrological and volcanological relationships are complex, but are not pertinent to the present considerations. We merely wish to document the highly heterogeneous nature of the ejecta.

Table 1 Analyses of mixed tephra

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
SiO ₂	73.72	50.98	69.61	54.1
Al ₂ O ₃	12.92	13.81	14.14	16.6
TiO ₂	0.83	1.89	0.38	0.8
FeO	3.12	13.31	2.89	8.2
MgO	0.57	6.18	0.36	3.3
CaO	2.14	10.82	2.00	8.0
MnO	0.07	0.30	0.08	0.1
Na ₂ O	3.59	2.47	4.78	5.0
K ₂ O	2.53	0.30	3.07	1.3
P ₂ O ₅	0.12	0.21	0.07	—
H ₂ O	—	—	3.49	2.4
Total	99.61	100.27	100.87	99.8

a. Rhyolitic glassy fiamme from welded part of layer *c*, Askja (average of 7 analyses); *b*. Basaltic glass, Askja (average of 5 analyses); *c*. Rhyodacite pumice, Santorini; *d*. Basaltic andesite scoria, Santorini. Analyses *a* and *b* were made on the electron microprobe. Analyses *c* and *d* are X.R.F. whole rock analyses (analyst A. Bond).

On the volcano Santorini several of the major acid plinian pumice fall deposits exposed in the caldera walls are mixed. Bond and Sparks¹⁰ recognised the following sequence of products in the Bronze Age Minoan eruption: plinian pumice fall deposit, base surge deposits, mud-flow deposits and ignimbrite. The bulk of the ejecta is rhyodacite containing 10–12% phenocrysts and there is no marked major element variation throughout the sequence. However, the upper half of the plinian deposit and the lower half of the base surge deposits contain vesicular, grey rounded pumice clasts (0.1–5 cm diameter) of basaltic andesite bulk composition (Table 1: analyses 3 and 4), which contain 25 to 35% phenocrysts set in a pale vesicular glass containing abundant quench crystals. The two types are quite distinct (SiO₂ 69.6 and 54.1%) and occur together in the same pumice clast. The tephra in the ignimbrite and lower part of the plinian deposit is not mixed.

Similar relationships are observed in the thick, prehistoric rhyodacitic pumice deposit (Bu) of the Middle Pumices Series¹¹. This deposit has two fall units, the lower one containing white rhyodacitic pumice only. In the upper fall unit grey, olivine bearing basaltic andesite pumice clasts, similar to the Minoan examples, are found intimately mixed with white rhyodacitic pumice. The proportion of basic tephra increases markedly upwards reaching an estimated 30% at the top of the upper fall unit. There are at least 12 acid pumice fall deposits exposed in the caldera walls of Santorini, which we have examined. Seven of these show field evidence of a mixed nature, with

relations between the two components similar to those described in the Minoan and Bu pumice deposits. The more basic component is not necessarily basaltic or indeed greatly different from the acid component: in one deposit the two components have SiO₂ contents of 61.1 and 65.7% respectively.

In these examples from Santorini and Askja mixing of the magmas can only have occurred shortly before eruption. In all cases the basic clasts are still dominantly glassy even in inclusions of a few millimetres size. Such inclusions could not survive in their acid host for any substantial time without either completely crystallising or dissolving by diffusion. In the case of Santorini, radiocarbon dates¹¹ indicate that periods between explosive acid eruptions are of the order 10⁴ yr. The mixing of the magmas can be shown, however, to have occurred no more than a few years before eruption and we believe that periods of weeks are more realistic for the time interval between mixing and eruption.

The interdiffusion of ions between two miscible silicate glasses can be estimated from:

$$l = \beta\sqrt{(Dt)} \quad (1)$$

where *l* is the length to which a diffusional wave penetrates from one area to another in a time *t*, *D* is the diffusion coefficient, and β is a constant which is conveniently 1.0 for 95% of the final equilibrium concentration¹². In the case of a sphere of basaltic glass, the length *l* may be set equal to the radius; for a radius 0.1 cm and for $D = 10^{-9} \text{ cm}^2 \text{ s}^{-1}$, which is typical of ionic diffusion rates at high temperature in silicate melts¹³, *t* is 3.85 months. In the Minoan deposits and Askja deposit, inclusions of basic material of 0.1 cm show little evidence of substantial chemical exchange with their host. We believe that the magmas were intermingled physically, but were erupted before any substantial chemical interaction took place. On Santorini we have observed the same relationships in several deposits and the eruptions clearly coincide with mixing events.

Model for magma mixing

We propose that magma mixing is a process which is likely to trigger an explosive eruption and that the features we have described in Askja and Santorini fit such a model. An acid magma emplaced in the upper crust is a slow moving and slow cooling body. Changes in physical conditions such as temperature and gas pressure will occur gradually, as crystallisation and conductive heat loss proceed, over periods of 10³–10⁵ yr (ref. 14). Gradients of temperature and physical properties will be small and rates of change slow. Intrusion of basic magma into the base of an acid magma chamber would dramatically alter this situation and produce an unstable, non-equilibrium system.

Studies of intrusive net-vein complexes³ show that the basic magma often forms pillow-like accumulations when intruded into acid magma. The temperature contrast between basaltic magma (1,100–1,300 °C) and acid magma (750–950 °C) is substantial. The break up into pillow-like units and inclusions results in an increased rate of heat transfer. The time *t* for a thermal wave to penetrate an object radius *R* can be estimated from

$$t = R^2/8K \quad (1)$$

where *K* is the thermal diffusivity. For a typical pillow 1 m diameter and with $K = 3 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ (ref. 14) the value of *t* is approximately 29 h. A basic magma that breaks up into metre or decimetre sized units equilibrates thermally with its immediate surroundings in periods of hours or days. Thus the acid magma host would be locally substantially superheated with extreme rapidity.

Magma mixing is believed to lead to vigorous convection in the acid magma. The superheated acid magma at the base of the chamber becomes less dense than the cooler magma above.

The condition for a convective cell to form is when the Rayleigh number (Ra) exceeds 660 (ref. 15) where

$$Ra = \alpha (\Delta T) d^3 \rho g / K \nu$$

In this expression α is the coefficient of thermal expansion ($= 5 \times 10^{-5} \text{ deg}^{-1}$), ν the viscosity, d the length scale of the chamber, K is the thermal diffusivity, ρ is the density of the acid magma, g is the acceleration due to gravity, and ΔT is the difference in temperature of the two magmas. Substituting values of viscosity (10^6 P), d for a magma in a mixing situation (10–1000 m) and temperature difference (10–300 °C) into the above equation yields Rayleigh numbers of 4×10^5 to 10^{13} . The values are orders of magnitude greater than the critical value.

In a simple model of a two-dimensional laminar convection cell¹⁶ the approximate expression for the vertical velocity in the cell is

$$V = (0.25(Ra)^{2/3}K)/d$$

Substituting the range of Rayleigh numbers estimated above, the vertical velocities range from 3.5 m d^{-1} to $3,000 \text{ m d}^{-1}$. These calculations suggest that magma mixing would produce vigorous and rapid stirring of the acid magma in periods of only days or weeks. The calculations are invalid if convection is turbulent, in which case stirring of the two magmas would be even more rapid. Shaw¹⁴ predicted from the work of Lighthouse¹⁵ that at values of Ra greater than 10^4 a magma would undergo fully turbulent convection. Our estimates of Ra indicate that fully turbulent convection is possible in a magma mixing event.

The likelihood of an eruption is greatly increased by magma mixing and convection. The acid magma is mobilised and magma at the base of the chamber is transported to the top. Our calculations indicate this takes place so rapidly that the magma is completely out of equilibrium with its surroundings. If the magma is saturated with volatiles at the base it will become highly supersaturated as it rises and gas pressures will rapidly build up. Increase of temperature in the acid magma reduces the solubility of gases such as water, thus providing a second, independent mechanism for supersaturating the magma. The volume increase due to the intrusion of basic magma is likely to increase the fluid pressure of the acid magma on its chamber walls, and fractures may form providing a pathway for the acid magma to the surface.

At the same time as the acid magma is superheated, the basic magma is quenched and some crystallisation may occur, resulting in exsolution and transfer of volatiles from the basic to the acid magma. Heating of the acid magma reduces its viscosity and ascent of the acid magma through fractures under a constant pressure gradient becomes more rapid and convection more intense as viscosity is lowered. Figure 2 illustrates schematically the model we envisage.

Triggering of acid explosive eruptions by magma has been observed in two geological settings. In regions of rifting such as Iceland magma mixing is the consequence of the fortuitous intersection of basaltic dykes with bodies of rhyolitic magma within acid volcanic centres. Basaltic injections may principally occur during regional rifting episodes such as the volcanotectonic event in the north-eastern volcanic zone of Iceland from 20 December 1975 to the time of writing¹⁷.

A similar rifting episode occurred in this region from 1874–1875 (ref. 17). The 1875 Askja eruption was preceded by earthquakes and minor activity for a few months before the main event. This rifting episode was accompanied by injection of basaltic dykes as evidenced by the basaltic Sveinagja fissure eruption of 18 February 1875 north of Askja. We suggest that one or more basaltic dykes intercepted the Askja magma chamber (Fig. 2), so that basaltic magma was injected into rhyolitic magma no more than a few weeks or months before the plinian eruption of March 1875.

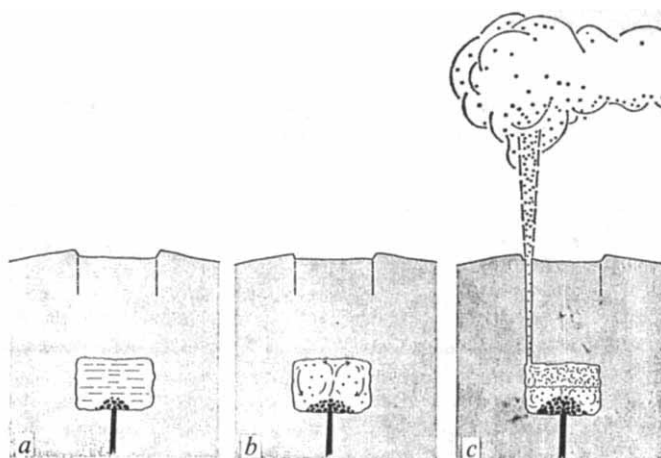


Fig. 2 Schematic model of the mechanism of triggering explosive eruptions by magma mixing. *a*. Basic magma is intruded into the base of acid magma. *b*. Pillows are formed and local superheating of the acid magma induces vigorous convection and vesiculation. *c*. The combination of intrusion of basic melt and vesiculation results in an increase in the fluid pressure of the magma system on the confining walls. Fractures form above the magma and an eruption is triggered.

The convection caused by mixing resulted in entrainment of small pieces of basic magma in the acid magma. These became thoroughly mixed throughout the chamber and so accounted for the simultaneous eruption of rhyolitic tephra with minor amounts of basaltic and mixed tephra from the beginning to the end of the eruption (Fig. 2*b*). At the end of the eruption the level of the magma had fallen to the base of the chamber where the size of basaltic units within the acid magma increases (Fig. 2*c*).

In island arc volcanoes, magma mixing may occur while magma enters from source regions into high level magma chambers. Anderson¹⁸ has provided convincing evidence that some andesite lavas are examples of magmas where both physical and chemical mixing have occurred well before eruption. On Santorini the field evidence is consistent with a small proportion of basic magma being mixed with large volumes of acid magma without substantial chemical interaction shortly before eruption.

In the model (Fig. 2) it is entirely possible that a large part of the basic magma is never erupted. Being considerably denser than their acid host large pieces of basaltic magma will remain at the base. Only inclusions of sufficiently small size will be entrained and mixed throughout the magma. Basic magma may induce convection in acid magma and eruption without being ejected itself. The possibility exists that magma mixing may have a role in eruptions other than those for which there is direct evidence of mixing.

Injection of basic magma into an acid magma chamber results in rapid superheating and induces convection of the acid magma, resulting in entrainment and physical mixing of some of the basic magma. Superheating and decompression of parts of the acid magma due to convection leads to supersaturation of volatile phases and vesiculation. The injection of basic magma and vesiculation of the acid magma markedly increases the pressure exerted by the acid magma on the confining walls. We believe that this pressure increase may often be sufficient to fracture the volcanic edifice and thus trigger an explosive eruption.

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Note added in proof: Another possible consequence of mixing of magmas is water supersaturation due to resorption of calcic plagioclase into the rhyolitic melt, which has been demonstrated experimentally by Yoder¹⁹.

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Magnetostratigraphy of the Cretaceous–Tertiary boundary in the San Juan Basin, New Mexico

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Magnetostratigraphy of a terrestrial sedimentary sequence in the San Juan Basin reveals that the Cretaceous–Tertiary boundary, recognised by the highest stratigraphic occurrence of dinosaurs, is younger than the midpoint of marine magnetic anomaly 29. This boundary is slightly younger than, but very close to, the Cretaceous–Tertiary boundary in the marine sedimentary sequence at Gubbio, Italy, which has recently been proposed as the magnetostratigraphic type section for the Upper Cretaceous and Palaeocene.

FAUNAL succession provides the major basis for recognition of geologic-time units. Boundaries between geologic-time units are placed at points of significant change in the fossil record, such as extinction of major elements of the fauna (here we use 'geologic-time boundary' in the practical sense of boundaries between biostratigraphic units recognised as belonging to different time intervals, but see ref. 1). These boundaries are generally considered to be globally synchronous. Geologic-time units and boundaries are generally defined in marine sequences. These units and boundaries must, then, be correlated with terrestrial sequences on the basis of other criteria. For example, the Cretaceous–Tertiary boundary is defined by extinction of invertebrates (primarily ammonites) in marine sequences but is recognised in terrestrial sequences by the last occurrence of dinosaur fossils. Thus, the global synchronicity of geologic-time boundaries has never been demonstrated. The resolution of this matter has implications for correlation of faunal changes in marine and terrestrial environments, as well as for geochronology itself.

Isotopic dating of horizons within various marine and terrestrial sequences has greatly refined knowledge of age limits and synchronicity of many geologic-time boundaries, but these techniques are not sufficiently precise to resolve the question of global synchronism. To test synchronicity of geologic-time boundaries, a globally synchronous phenomenon must be recorded in both marine and terrestrial sedimentary sequences. Geomagnetic polarity reversals are globally synchronous phenomena² and provide a technique for testing synchronicity of geologic-time boundaries.

Determination of palaeomagnetic polarity stratigraphy in sequences of marine and terrestrial sediments bracketing a particular geologic-time boundary can provide the data necessary to reveal the synchronism or lack of synchronicity of that boundary.

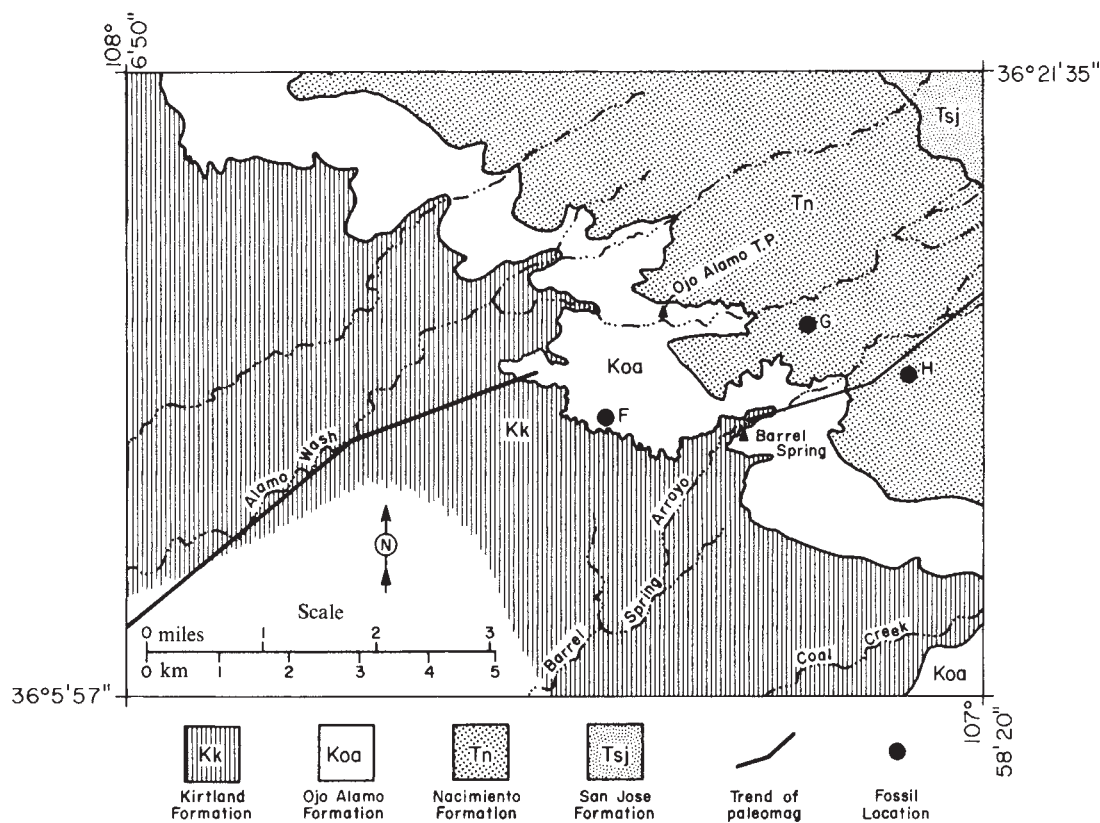
Significant changes occur in both invertebrate and vertebrate faunas at the boundary between the Cretaceous and Tertiary Periods (also Mesozoic and Cainozoic Eras). Through the Deep Sea Drilling Project (DSDP), the earliest foraminiferal zones deposited on oceanic crust of known magnetic anomaly number are providing valuable information on the alignment of invertebrate biostratigraphy with the magnetic anomaly time scale^{3,4}. As a result, considerable progress has been made in referring faunal changes at the Cretaceous–Tertiary boundary in marine sequences to a point in the geomagnetic polarity time scale. These data are complemented by palaeomagnetic polarity determinations on DSDP cores⁵. In addition, Alvarez *et al.*⁶ have proposed a magnetostratigraphic type section for Upper Cretaceous and Palaeocene in Gubbio, Italy. The Cretaceous–Tertiary boundary in this marine sequence occurs near the top of the reversed polarity interval preceding magnetic anomaly number 29.

The San Juan Basin, New Mexico and Colorado, contains a complete Upper Cretaceous into Lower Tertiary sequence of terrestrial sediments. We report here the results of a palaeomagnetic and palaeontologic investigation of the sediments embracing the Cretaceous–Tertiary boundary. These data are an important step toward resolution of the Cretaceous–Tertiary boundary in terrestrial sedimentary sequences, and its comparison with the Italian marine sequence is valuable in evaluating the global synchronism of this important geologic-time boundary.

Stratigraphy and vertebrate palaeontology

The San Juan Basin, located in northwest New Mexico and southwest Colorado, contains one of the most complete Palaeocene terrestrial stratigraphic successions in the world. The Palaeocene sediments are underlain by late Cretaceous and are overlain by early Eocene strata. The terrestrial sedimentary sequence in the San Juan Basin proceeds from shales and interbedded coal layers of the Fruitland Formation, through shales, siltstones and sandstones of the Kirt-

Fig. 1 Trend of the palaeomagnetic section and location of vertebrate fossils. Distribution of geological formations is after Bauer¹². Fossil localities: F, Ojo Alamo local fauna; G and H, *Ectoconus* and *Taeniolabis* zones, respectively, of the Puercan Land Mammal Age.



land Formation, and into conglomeratic sandstone and interbedded shale of the Ojo Alamo Formation. These dinosaur-bearing formations are overlain by varicoloured clays of the Palaeocene Nacimiento Formation and the Eocene San José Formation.

Primary reference faunas for Puercan (early Palaeocene) and Torrejonian (middle Palaeocene) Land Mammal Ages were collected from the Nacimiento Formation⁷. The Tiffanian Land Mammal Age (late Palaeocene) was based on a fauna collected from sediments in Colorado on the north side of the San Juan Basin. These late Palaeocene sediments are usually included in the San José Formation. On the east side of the basin, the San José Formation contains Wasatchian (early Eocene) mammals¹⁸.

The terrestrial sequence in the San Juan Basin is underlain by marine deposits of the Lewis Shale and the Pictured Cliffs Sandstone formations, which correlate with the middle of the Pierre Shale in the interior North American sequence⁵. This indicates that the lower limit of the San Juan Basin terrestrial sequence is equivalent to late Campanian or early Maastrichtian stages of the European Cretaceous succession.

Our palaeomagnetic section (Fig. 1) begins in Hunter Wash and extends to the head of Barrel Spring Arroyo. The lowest faunal level of the Hunter Wash section is the Hunter Wash local fauna⁹ in the Fruitland Formation. This fauna is represented by bone symbols at the 12 m and 33 m levels in the Hunter Wash section (Fig. 2). The Hunter Wash fauna includes fish, turtles, crocodiles, hadrosaur dinosaurs (*Parasaurolophus*), ceratopsian dinosaurs (*Pentaceratops*), carnosaur dinosaurs, multituberculate mammals (probably five genera), marsupial mammals (probably two genera)⁹, and placental mammals (probably two genera)⁹. The highest Cretaceous faunal level, Ojo Alamo local fauna, occurs in the Ojo Alamo Formation at the 256 m level of the Hunter Wash–Alamo Wash section (at F in Fig. 1). The Ojo Alamo fauna includes fish, turtles, crocodiles, hadrosaur dinosaurs (*Kritosaurus*), ceratopsian dinosaurs, carnosaur dinosaurs and sauropod dinosaurs (*Alamosaurus*)¹³. Unidentified mammals have been

found from the Ojo Alamo local fauna, but descriptions have not been published. The Hunter Wash local fauna may be correlated with the Edmontonian or Lancian Land Mammal Ages^{9,10}.

There has been much confusion surrounding the stratigraphic and age limits of the Ojo Alamo Formation^{9,11}. We accept the definition of Bauer¹², which includes the important Ojo Alamo fauna in the Ojo Alamo Formation^{9,13,14}. We also recognise the last occurrence of dinosaurs within the Ojo Alamo Formation. As emphasised by Baltz *et al.*¹⁵, the upper conglomerate with logs of the Ojo Alamo Formation intertongues with the base of the Nacimiento Formation in Barrel Spring Arroyo.

The lowest stratigraphic occurrence of Palaeocene mammals is the *Ectoconus* zone of Sinclair and Granger¹⁴, shown in our Barrel Spring Arroyo section (Fig. 3). The *Ectoconus* zone (G in Figure 1) and the overlying (by about 15 m) *Taeniolabis* zone (H in Fig. 1) are local divisions of the Puercan Land Mammal Age (early Palaeocene). The *Ectoconus* zone is characterised by the abundance of the periprychid condylarths *Ectoconus*, *Conacodon*, *Hemithylaeus* and *Anisonchus*, and the absence of the large multituberculate *Taeniolabis*. Fossils are not present continuously between the Ojo Alamo faunal level and the *Ectoconus* zone. Thus, the Cretaceous–Tertiary boundary in this terrestrial sequence occurs between these two faunal levels. Accordingly, the boundary is shown as a vertically striped region in Figs 4, 5 and 6.

Magnetostratigraphy

Three oriented block samples were collected at each of 212 palaeomagnetic sites. The nominal stratigraphic separation between sites was 3 m. Details of the collection technique are discussed in ref. 16. Remanence measurements were accomplished with a cryogenic magnetometer (Superconducting Technology, C-102), and alternating field (AF) demagnetisation was performed with a Schonstedt GSD-1 demagnetiser. The average intensity of the natural remanent magnetism (NRM) was $\sim 10^{-6}$ G. Although AF demagnetisation at peak fields of 200–300 Oe was necessary

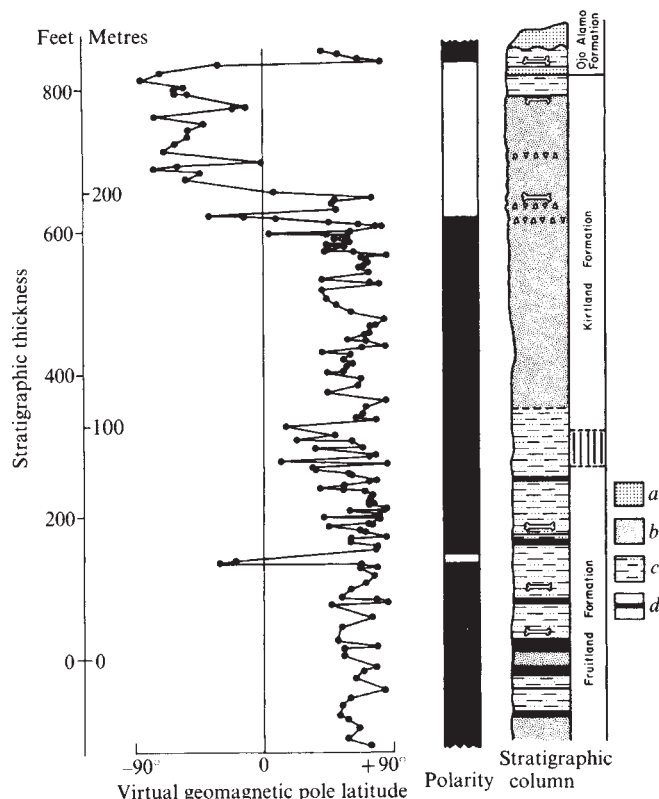


Fig. 2 Hunter Wash-Alamo Wash palaeomagnetic data. Site average virtual geomagnetic pole latitude, resulting polarity column and lithological column are plotted against stratigraphic thickness. Shaded regions in polarity column correspond to normal polarity, unshaded to reversed polarity. Palaeomagnetic samples were AF demagnetised in peak field of 200 Oe. Lines at Fruitland-Kirtland contact indicate the gradational nature of the contact. Bone symbols indicate important vertebrate fossil levels. *a*, Conglomeratic sandstone; *b*, sandstone; *c*, siltstone; *d*, coal layer.

to remove secondary components, unambiguous determination of the polarity of the primary depositional remanence was possible for almost all sites. As there is no accepted procedure for data rejection in palaeomagnetic work on sedimentary rocks of this type, we include all results. It should be recognised, however, that large normal components of viscous or chemical remanence can sometimes obscure or replace a primary reversed NRM. Thus, a certain amount of interpretation must take place in obtaining a polarity log from a set of data. Of particular importance in these interpretations are the motions of the NRM vectors during AF demagnetisation and the degree of clustering of the remanence vectors of the three samples at each site. A more complete discussion of the rock-magnetic properties will be published elsewhere.

Figure 2 shows the site average virtual geomagnetic pole (VGP) latitudes in the Hunter Wash-Alamo Wash section. Positive VGP latitudes indicate normal polarity while negative VGP latitudes indicate reversed polarity. But, incomplete removal of secondary normal components generally prevents VGP latitudes of reversed sites from reaching high southerly latitudes. There are two features of this polarity column which require comment. Although the short reversed polarity interval at the 40 m level is based on only two sites, we are confident this magnetozone is real because of the coherent motion of the NRM vectors during AF demagnetisation. There is some inconsistency of the data at the 190 m level near the bottom of a long reversed polarity zone. We place the bottom of this magnetozone at the lowest occurrence of a well-behaved site

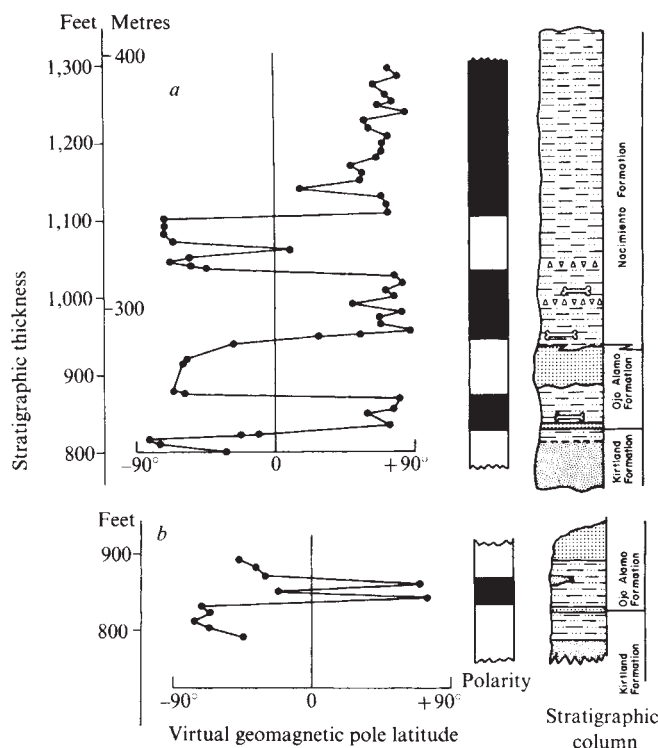
with negative VGP latitude. The stratigraphic interval at 190–200 m is coarse siltstone, and we believe it has been remagnetised by the Earth's present field during weathering of the outcrop.

The palaeomagnetic data for sections in Barnum Brown Amphitheater and Barrel Spring Arroyo are shown in Fig. 3. Again, two features of the polarity interpretation require comment. The site with negative VGP latitude in the middle of the normal polarity magnetozone in Barnum Brown Amphitheater was very weakly magnetised and moved toward positive VGP latitude during AF demagnetisation. Because of these observations and our opinion that magnetozones should not be defined by results at a single site, we favour the polarity column shown rather than a more literal interpretation of the data. The site at the 300 m level in Barrel Spring Arroyo consistently moved toward negative VGP latitude during demagnetisation. Thus, the primary NRM component is of reversed polarity.

The stratigraphic correlation required to construct a composite magnetostratigraphic column through the Cretaceous-Tertiary boundary is shown in Fig. 4. The three sections were lithologically correlated in the field by tracing the lower conglomerate layer and overlying clay layers of the Ojo Alamo Formation between the sections. The total lateral distance between the top of the Hunter Wash-Alamo Wash section and the bottom of the Barrel Spring Arroyo section is only 4 km. The beds are easily traced, and there is little chance of error in this lithological correlation. In addition, the bottom of the normal polarity magnetozone at the 260 m level also correlates between sections and confirms the lithological correlation.

Stratigraphers have generally placed an unconformity at the top of the upper conglomerate layer of the Ojo Alamo Formation and have placed the Cretaceous-Tertiary boundary at that unconformity. The Ojo Alamo and overlying Nacimiento Formation, however, intertongue in Barrel Spring Arroyo. Our section avoided the unconformity at the top of the upper conglomerate by sampling the

Fig. 3 Barnum Brown Amphitheater (*b*) and Barrel Spring Arroyo palaeomagnetic data (*a*). Lithological symbols are as in Fig. 2.



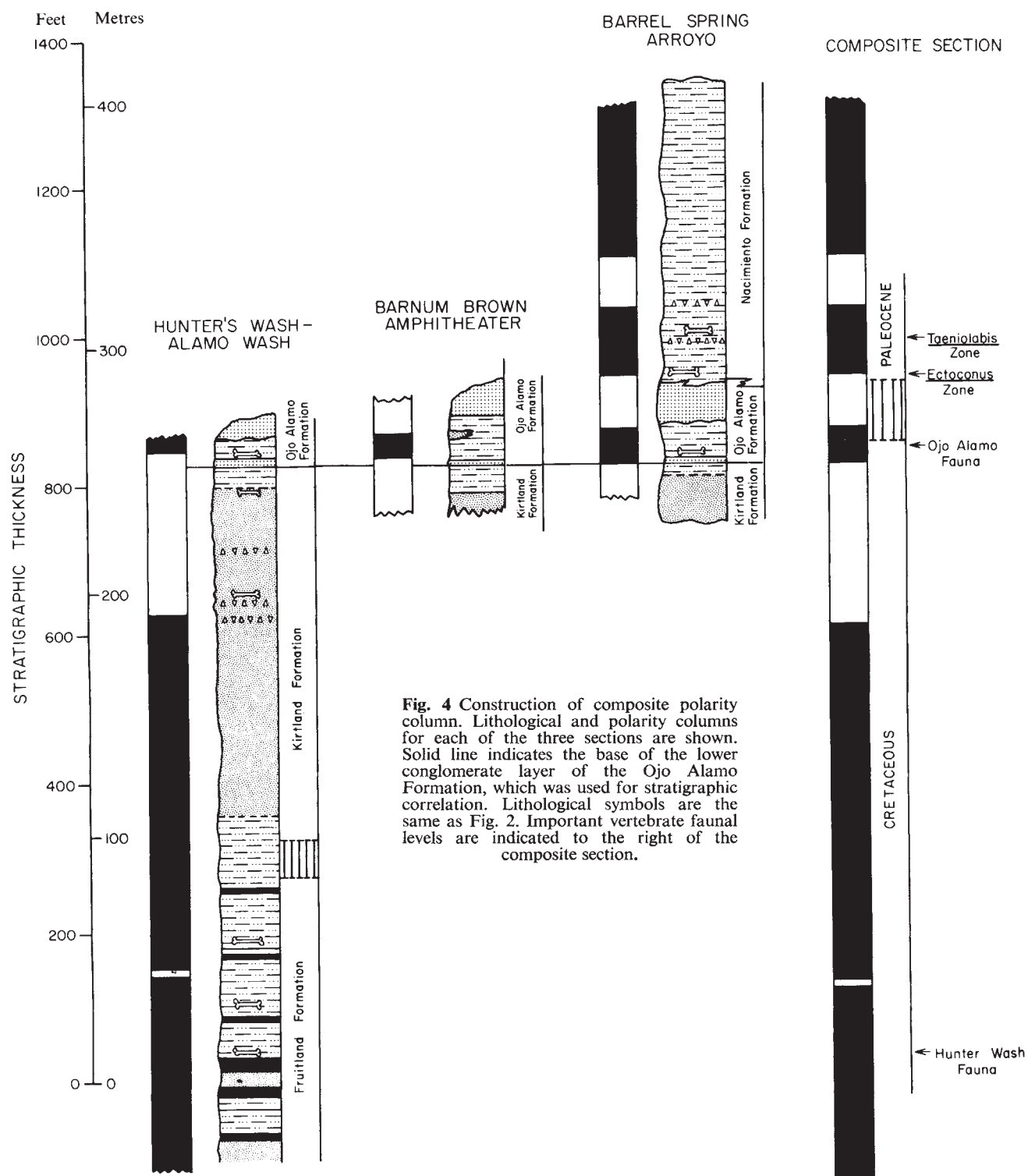


Fig. 4 Construction of composite polarity column. Lithological and polarity columns for each of the three sections are shown. Solid line indicates the base of the lower conglomerate layer of the Ojo Alamo Formation, which was used for stratigraphic correlation. Lithological symbols are the same as Fig. 2. Important vertebrate faunal levels are indicated to the right of the composite section.

Nacimiento Formation in the region of the intertonguing. Tertiary mammalian fossils from the *Ectoconus* zone have been recovered in Barrel Spring Arroyo above the intertonguing while dinosaur fossils occur below the intertonguing. Thus, we have sampled through a continuously exposed stratigraphic section with late Cretaceous and early Palaeocene faunas in superpositional order. We assert that the resulting composite magnetostratigraphic column is a virtually complete section through the Cretaceous-Tertiary boundary. The stratigraphic interval within which the Cretaceous-Tertiary boundary must be placed is shown in Fig. 4.

Another possible unconformity is at the base of the lower

conglomerate layer of the Ojo Alamo Formation. This layer is 2-3 m thick and varies from a homogeneous sandstone to a sandstone with basal conglomerate. There are three lines of evidence which indicate that the lower conglomerate does not represent a significant unconformity. First, the Ojo Alamo faunal zone includes the shales above and below the lower conglomerate. Second, in this region there is a very strong lithological similarity of the shale layers above and below the lower conglomerate. In fact, this similarity is so strong that Brown¹³ and Sinclair and Granger¹⁴ included the shales of the upper Kirtland Formation below the lower conglomerate in their definitions of the Ojo Alamo Formation. And third, as shown below,

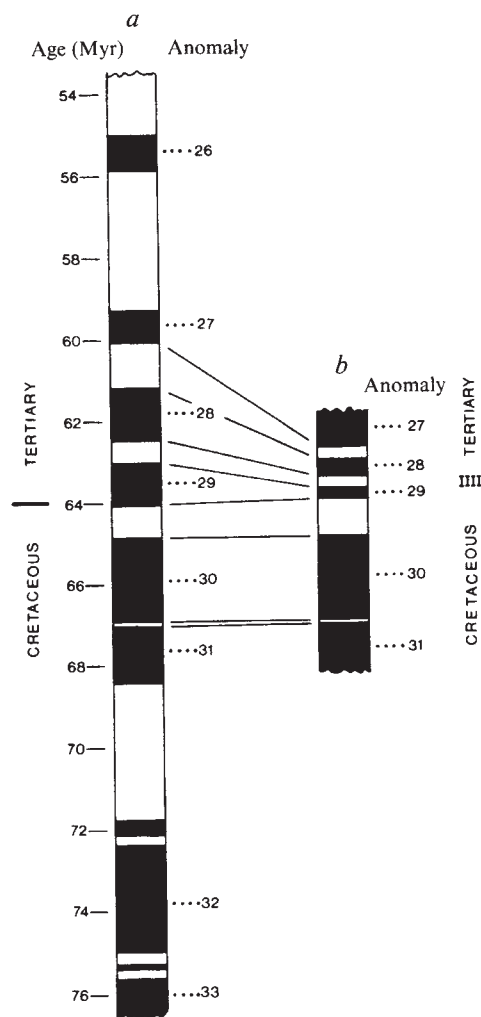


Fig. 5 Correlation of composite polarity column to marine magnetic anomaly time scale. A portion of the Tarling and Mitchell time scale¹⁷ is shown in *a*, while the composite polarity column from the San Juan Basin is shown in *b*. The San Juan Basin polarity column is scaled to make the length of anomaly 30 match the Tarling and Mitchell time scale¹⁷.

there is a very close similarity between the relative thicknesses of the magnetozones in the composite section from the San Juan Basin with the lengths of corresponding polarity intervals in the marine magnetic anomaly time scale. This similarity suggests that no significant unconformity exists at the base of the lower conglomerate of the Ojo Alamo Formation.

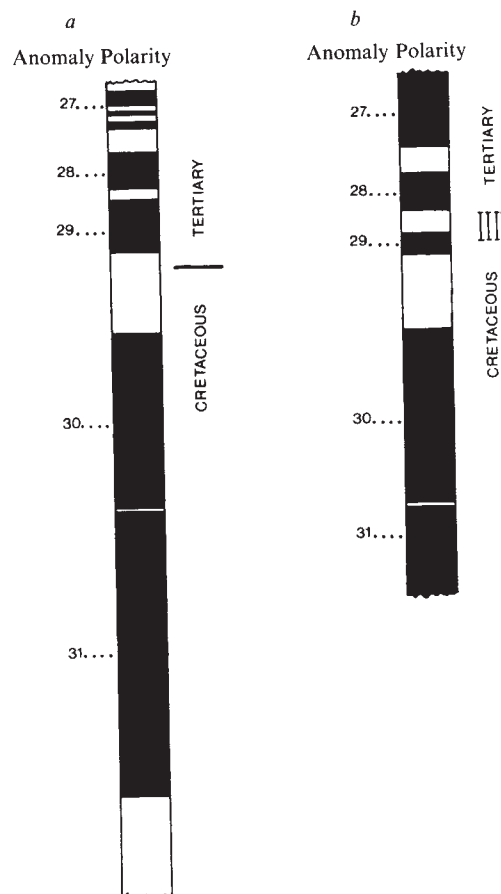
Discussion

Because the lower limit for the age of the Fruitland Formation (as determined from underlying marine formations) is late Campanian to early Maastrichtian, the composite magnetostratigraphic column from the San Juan Basin must be in the late Campanian to Palaeocene interval. Alvarez *et al.*⁶ have shown that late Campanian-early Maastrichtian time corresponds to magnetic anomaly number 33. Thus, none of the magnetozones from the San Juan Basin can be older than anomaly 33. The upper portion of our polarity column is Puercan (early Palaeocene). Evidence from both the magnetic anomaly time scale¹⁷ and magnetostratigraphic studies⁶ indicate that the uppermost magnetozones in our polarity column could not be younger than anomaly 26. Therefore, in attempting to correlate the magnetozones to our composite section with magnetic anomalies, we are constrained to the interval of magnetic anomaly numbers 26–33.

To correlate a magnetostratigraphic section with the anomaly time scale, the section must be of sufficient length and character to allow a unique correlation. A characteristic feature of the marine magnetic anomaly sequence is the very short reversed polarity interval between magnetic anomalies 31 and 30 (Fig. 5). Examination of the polarity sequence in the San Juan Basin reveals the same unusually long normal polarity zone containing one very short reversed polarity zone. In both sequences the long normal polarity interval is followed by more frequent, evenly spaced reversals in the Lower Tertiary. Given the constraints outlined above, the sequence of magnetozones in our composite section contains sufficient character to allow correlation with the magnetic anomaly time scale. The resulting correlation (Fig. 5) indicates that the normal polarity magnetozones in the San Juan Basin section correspond to magnetic anomalies 27 to 31. No other correlation with the magnetic anomaly time scale can be made without assuming very large and rapid changes in sedimentation rate.

Figures 4 and 5 demonstrate the position of the Cretaceous-Tertiary boundary within our stratigraphic sequence. The highest occurrence of in-place dinosaur fossils is toward the top (young side) of anomaly 29 while the lowest Palaeocene mammal fossils are found near the top of the succeeding reversed interval between anomalies 28 and 29. Therefore, the Cretaceous-Tertiary boundary in this North American terrestrial sedimentary sequence occurs in the interval between the middle of anomaly 29 and the beginning of anomaly 28.

Fig. 6 Correlation of magnetozones in *b*, the San Juan Basin and *a*, the marine Scaglia Rossa Limestone of Gubbio, Italy⁶. Only the upper portion of the polarity column from Gubbio, Italy, is shown. The polarity columns were scaled so that the lengths of anomaly 30 are equal.



A correlation of the Gubbio, Italy section with the San Juan Basin section is shown in Fig. 6. This correlation is a graphic illustration of the potential of magnetostratigraphy. Through the intermediate step of correlating these sections to the magnetic anomaly time scale, a very accurate stratigraphic correlation between a marine sequence in Italy and a terrestrial sequence in western North America has been accomplished.

Our data show that the Cretaceous-Tertiary boundary in marine and terrestrial rocks is not synchronous. The Cretaceous-Tertiary boundary, recognised by vertebrate fossils in the San Juan Basin, is younger than the Cretaceous-Tertiary boundary, as recognised by marine invertebrate fossils. The amount of non-synchronicity is small, however. The age difference between the beginnings of anomalies 29 and 28 is of the order of 1.5 Myr (ref. 17). Thus, even the maximum time difference between the Cretaceous-Tertiary boundary at the Gubbio, Italy section and at the San Juan Basin section is probably less than 1.5 Myr. The minimum time difference may be as little as 0.5 Myr. Considering the vast geologic time distance from which we are viewing this event, the relative degree of synchronicity is remarkable.

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New geochronologic and palaeomagnetic data for the hominid-bearing Hadar Formation of Ethiopia

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A 2.6 Myr K/Ar age has been derived for a primary unworked tuff high in the hominid-bearing Hadar Formation (Kada Hadar Member), stratigraphically above all the important fossil finds. A 2.6 Myr fission track age has been derived on zircons from this tuff. New K/Ar results on the Kadada Moumou basalt (Sidi Hakoma Member) suggest a 3.0 Myr age. Preliminary interpretation of a detailed continuous palaeomagnetic section through the formation indicates the existence of persistent normal and reversed sequences. With the radiometric age control this magnetic sequence appears to correlate with the Gauss Epoch. These initial results imply the fossil-rich Hadar Formation spans from somewhat older than 3.1 Myr to somewhat younger than 2.6 Myr.

THE well-exposed Plio-Pleistocene Hadar Formation^{1,2} in the central Afar Depression is of lacustrine and lake-margin

origin with a rich vertebrate fauna including relatively abundant and excellently preserved hominid material³⁻⁵. In this paper, we present new geochronologic and palaeomagnetic data pertinent to establishing the age of the formation. A geologic column for the Hadar Formation, which represents the area exposed along the Sidi Hakoma and Kada Hadar tributaries north of the Awash River, is shown as section A (Fig. 1). Details of the stratigraphy are presented elsewhere^{1,2}.

Geochronology of Bouroukie Tuffs

Three tuffs have been newly found in the Kada Hadar Member (KHM), which is the uppermost member of the Hadar Formation. We have named these tuffs BKT₁, BKT₂ and BKT₃, from oldest to youngest (Fig. 1), which are well exposed in the Bouroukie tributary to the Kada Hadar drainage. BKT₁ is a 15-cm thick chalky white clay typical of most of the Hadar Tuffs and probably represents completely altered glass ash. BKT₃ is a 75-cm thick buff-coloured glass shard ash with numerous tubular fillings presumed to be root casts. BKT₂ is at present the most

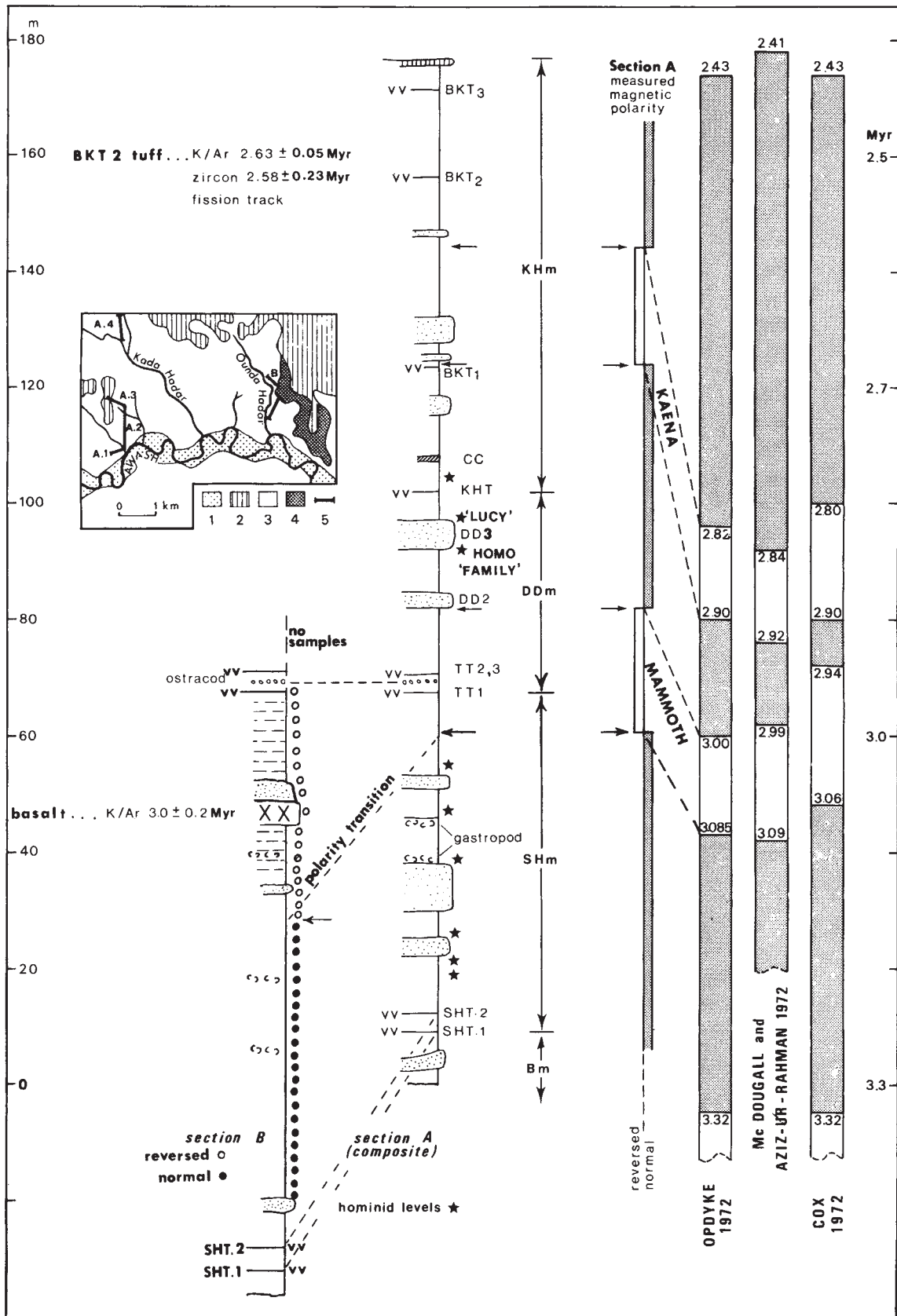


Fig. 1 Palaeomagnetic log of the Hadar Formation. Location of the Gauss Normal Epoch containing the Mammoth and Kaena Reverse Events is shown with reference to three recent versions of the geomagnetic polarity time scale. The important hominid levels are indicated, including the australopithecine skeleton 'Lucy' and the Homo family (site 333). Also shown are all of the tuffs and the basalt and the dates for BKT₂ and the basalt. The members of the Hadar Formation are from the bottom up: Basal Member (BM); Sidi Hakoma Member (SH); Deden Dora Member (DD); and Kada Hadar Member (KH). Legend for the generalised geologic inset map: 1, recent Awash River alluvium and forest; 2, Pleistocene capping gravels; 3, Hadar Formation; 4, Kadada Moumou basalt; and 5, location of palaeomagnetic sections. In palaeomagnetic section B, the open circles schematically represent reversed section and the solid circles represent section of normal polarity.

promising of all the tuffs in the Hadar Formation for geochronology. It consists of two beds separated by 1 m of brown silty clay. The lower bed is a 10–15 cm thick pale yellow crystal-bearing tuff. It is similar to the lower part of the 40-cm thick upper bed on which our geochronology has focused.

The petrology of the upper BKT₂ tuff bed indicates it is of primary pyroclastic origin, uncontaminated by older sediment. The bed has been sharply stratified into two layers by primary aeolian differentiation during the explosive eruption. The upper layer making up two-thirds of this bed is a porous black and white fine lapilli lithic tuff consisting entirely of 1–4 mm grains and granules of rock fragments partially cemented by calcite. The lower layer making up one-third of the bed is a pale yellow crystal and lithic tuff which provided anorthoclase for K/Ar analyses and zircon for the fission-track dating.

The upper lithic tuff layer consists of 65% smaller grains of a brown glass obsidian that is very vesicular (30–50% holes), and 35% larger grains and granules of white (clear) pumice with a spindle-like alignment of glass fibrils. A gas-rich eruption that became explosive is indicated by these grains, and their coarseness suggests the source was one of the nearer-by Afar acidic volcanoes. Very fine grained basalt (?) forms uncommon inclusions in the obsidian. Both obsidian and pumice have large uncommon phenocrysts of anorthoclase and pyroxene similar to the crystals we will describe from the lower layer. The preservation of fragile broken bubble projections on the obsidian fragments indicates that very little or no rolling transportation occurred. That there was no sedimentary reworking is also indicated by the preservation of the primary aeolian differentiation layering, and the complete lack of any matrix of finer normal density grains for the lower density framework grains of vesicular obsidian and pumice.

The lower one-third layer obtains its pale colour and friability because of the entirely clay-altered 0.5–1 mm grains of collapsed pumice which make up 70% of the layer. Five per cent of the layer is fragments of a magnetite-rich non-vesicular brown obsidian and lesser amounts of a magnetite-rich fine-grained basalt (?). The remaining 25% of the layer is euhedral to subhedral crystals of an exceedingly simple volcanic mineral assemblage: about 24% of a very low-K₂O anorthoclase; 1% green augite rimmed by fibrous amphibole (?); and common magnetite and zircon. All of these four are cogenetic as indicated by the distinctive occurrence of the magnetite as euhedral octahedrons in isolated grains and as very frequent inclusions in each of the other three minerals, and by the occurrence of binary combinations among all the minerals. In addition, the minerals are cogenetic with the obsidian and pumice rock fragments in the upper layer because of their occurrence there as large phenocrysts. It is unlikely the crystals were derived by explosion of a pre-existing rock because all grains are free of any adhering rock matrix. The anorthoclase appears milky under binocular microscope, but clear and unaltered in thin section. It is optically unusual for a potassium feldspar in displaying to various degrees the three major simple feldspar twins (albite, pericline and carlsbad) as well as being untwinned. For example, carlsbad twinned grains occur with one half albite twinned and the other untwinned and optically anorthoclase. Because some grains only display albite twinning, the presence of some plagioclase cannot be ruled out. Staining of the thin section for K₂O, however, developed an equally light measles-like stain on all grains independent of the twinning characteristics, indicating each of the grains is of the same moderately low K₂O content. Additionally suggestive that only one feldspar is present comes from K₂O analyses of seven finely resolved density fractions of the total feldspar. The total range in K₂O was only 1.70–2.00%, with no variation in the four lightest sinks. Thus, no high K₂O feldspar phase exists

in the rock and probably no plagioclase, just the single low-K₂O anorthoclase.

Because of the simple and distinctive mineral assemblage, any extraneous sedimentary components in the lower layer should be apparent. The complete absence of quartz, high-K₂O feldspar, and any variety of rock fragments, and the probable absence of plagioclase, sensitively indicates no admixture of older sedimentary grains has occurred. Together the mineralogy, texture and structure provide abundant evidence that the BKT₂ upper tuff bed is of primary pyroclastic origin with crystals formed at the time of eruption and uncontaminated by older material thereafter.

Four repeat potassium and argon measurements on splits of the same unpicked separate of anorthoclase yield consistent analytically precise ages of 2.65 ± 0.05 Myr (80% radiogenic argon) (Table 1). We report the CWRU laboratory average of 14 measurements of the USGS LP6 Biotite interlaboratory standard because the tracer constant for our bulb-pipette tracer system is presently assigned a value that causes all our age measurements to be 3.5% low in comparison with the average of other laboratories which have run this standard (J. C. Engels, personal communication). Correcting to these other laboratories would raise the 2.6 Myr age to 2.7 Myr. This discrepancy is being evaluated by measuring absolute atmospheric argon standards.

The zircon, which is a common accessory in this sample, has a large range in grain size, from 30 to 120 μ m. All sizes uniformly have a light reddish cast and are euhedral pyramidally terminated prisms. The length to width ratio is variable from 2–10, with stout forms predominant. Eleven grains of the +140 mesh fraction were dated by one of us (C.W.N.) by counting fossil and induced fission tracks (Table 2). The average fission track age of the eleven grains is 2.58 Myr with a standard deviation of ± 0.23 Myr.

Because all of the Hadar Formation fauna recovered so far is stratigraphically below BKT₂, these concordant K/Ar and zircon fission track ages, and the unlikelihood that the tuff is contaminated, indicate that the fauna is older than 2.6 Myr.

Geochronology of Kadada Moumou basalt

The stratigraphic position of the Kadada Moumou basalt flow at the eastern edge of the Hadar site is now established within the upper part of the Sidi Hakoma Member. The Kadada Moumou subplateau is upheld by the 1–4 m thick resistant basalt flow which is capped unconformably by mid-Pleistocene (?) gravels and sands with Acheulean artifacts⁶. Near the northwest end of the basalt some grey mudstone above the basalt may represent the only Hadar Formation sediment not stripped by erosion from above the basalt. The basalt is 70 m above the Sidi Hakoma Tuff (SHT) which forms the base of the Sidi Hakoma Member. The basalt was erupted on a gentle erosional surface of subdued topography with local relief up to about 10 m. The northwest end of exposure of the basalt probably represents a slightly eroded flow front. A cross-section of a large 10 m deep palaeo-channel or palaeo-embayment is exposed along this flow front. We have established that the channel is filled with Hadar-age sand beds which post-date the basalt and which can be related to the overlying Hadar sequence. The channel fill sand beds have original dips parallel to the approximate 20° slope leading downward from the basalt. The initial sand bed in the channel contains numerous boulders and cobbles of the vesicular basalt and thus post-dates the basalt. These channel-fill sands are of undoubted Hadar Formation age because they contain abundant Hadar fauna. One basalt boulder in the basal sand of the channel was found within the metre-wide carapace of a fossil turtle (discovered by B. T. Gray). Conformably above the channel fill is a distinctive extensive sand horizon with abundant root casts. It can be traced from the basalt front directly

into the main Hadar sequence, where it lies 20 m below the triple tuff marker horizon that is the top of the Sidi Hakoma Member (SHM) (Fig. 1). This marker has been walked out to the main fossil bearing localities in the Kada Hadar drainage. Thus, the basalt erupted on an erosional surface that is probably minor within the Sidi Hakoma Member at the eastern edge of the Hadar site. In the Kadada Moumou area, it is located 70 m above the base and 20 m below the top of the member. Further west in the main part of the Hadar site the Sidi Hakoma Member has a total thickness of only 60 m (Section A) compared to the 90 m in the east. A more detailed correlation of the

The average of six K/Ar analyses of three geographic samples of the shiny black variety is 3.0 Myr with an average deviation of ± 0.06 Myr (Table 1), including our two previously reported results¹. In contrast, one sample of the more altered sugary-textured variety gives an age of 2.65 Myr (two analyses) and another sample, which is a little more altered, an age of 2.50 Myr. Each individual analysis of the Kadada Moumou basalt has an estimated uncertainty of ± 0.2 Myr, because the extracted argon was only 10–20% radiogenic.

A key factor in interpreting the K/Ar discrepancy between the two varieties is secondary mineralisation, which

Table 1 K/Ar Data

Sample	Weight (g)	⁴⁰ Ar* per g	⁴⁰ Ar*/ ⁴⁰ Ar (×100)	%K ₂ O†	K–Ar age (Myr)‡
<i>a</i> , BKT ₂ -tuff					
38–75–1 Anorthoclase					
35/80 mesh					
no. 1§	5.4	0.0774	84.8	2.00 ± 0.01(2)	2.62 ± 0.08
no. 2	3.8	0.0740	76.8	1.96 ± 0.004(2)	2.55 ± 0.08
no. 3	4.4	0.0784	67.1	1.96 ± 0.004(2)	2.70 ± 0.08
no. 4	4.2	0.0766	79.8	1.96 ± 0.004(2)	2.64 ± 0.08
					2.63 ± 0.04(4)†
<i>b</i> , Kadada Moumou Basalt					
(1) Massive black phase					
KM					
1–73 ₁ ¶	10.0	0.0291	20.6	0.657 ± 0.008(11)	3.00 ± 0.20
1–73 ₂ ¶	10.0	0.0291	15.9	0.657 ± 0.008(11)	3.00 ± 0.20
2–74 ₁ ¶	10.0	0.0318	15.2	0.755 ± 0.008(9)	2.95 ± 0.20
2–74 ₂	10.0	0.0326	13.3	0.755 ± 0.008(9)	2.90 ± 0.20
4–74 ₁	10.0	0.0318	13.9	0.68 ± 0.01(5)	3.15 ± 0.25
4–74 ₂	10.0	0.0308	13.0	0.68 ± 0.01(5)	3.05 ± 0.25
					3.00 ± 0.05(6)†
(2) Sugary grey phase					
KM					
1–74	10.0	0.0326	13.3	0.727 ± 0.008(5)	2.50 ± 0.25
3–74 ₂	10.0	0.0295	25.8	0.747 ± 0.005(5)	2.65 ± 0.20
3–74 ₂ **	10.0	0.0298	14.4	0.747 ± 0.005(5)	2.70 ± 0.25
LP 6 Interlaboratory biotite standard	0.24†	18.44 ± 0.36 (19)†	95 (19)	10.14 ± 0.08(18)	

* Radiogenic argon, 10^{-10} mol g⁻¹.

† Average and average deviation, no. of measurements in parentheses.

‡ ⁴⁰K/⁴⁰Ar total = 1.19×10^{-4} ; $\lambda_b = 4.72 \times 10^{-10}$ yr⁻¹; $\lambda_e = 5.85 \times 10^{-11}$ yr⁻¹; age uncertainty for individual samples calculated using 0.3% uncertainty in isotopic composition of sample and atmospheric argon, a 1% uncertainty in argon spike, 1% uncertainty in K₂O, and 1% uncertainty in sample homogeneity.

§ Treated, 4% cold HF 1 minute, resieved to +100 mesh.

|| Low temperature fusion within a previously fused and degassed basalt.

¶ Analysis previously reported, ref. 1.

** Sample not pre-baked before extraction.

basalt position within this thinner section to the west is suggested in the discussion on magnetostratigraphy (Fig. 1).

Several K/Ar results on the basalt fall into two groups which correlate with the two subtle varieties of the flow selected from fresh cores of large talus blocks. These varieties probably represent a different vertical position in the flow not yet observed. One variety is a relatively unaltered dark black rock breaking with a smooth surface compared to a dark grey more granular or sugary-surfaced variety which thin section study indicates is relatively more altered. Both types consist of fine-grained plagioclase, augite, magnetite and minor olivine in a cryptocrystalline glassy matrix. The dark grey granular variety is coarser grained (0.2–0.4 mm compared with 0.15 mm), and contains much less of the cryptocrystalline matrix (10% compared with 35%) than the smooth black variety. The smooth black variety largely obtains its character from this abundant cryptocrystalline matrix, charged with magnetite dust.

occurs in two ways: (1) a filling of cavities, which occurs in both varieties of basalt and is interpreted not to have affected the age measurement; and (2) a yellowish clay alteration of the glassy matrix which affects only the grey variety and could well have caused argon loss. As regards the secondary filling, both varieties have a diktytaxitic texture (angular interstitial cavities) with the smaller cavities filled with a yellowish clay (?) and larger cavities lined with the clay concentric about a coarse calcite filling. This secondary filling is the same observed in the abundant vesicles at the top of the flow which may be empty, lined with clay, or lined with clay around calcite. A complete gradation can be observed in the same outcrop between samples with only fine angular interstitial cavities and samples with only normal rounded vesicles. As regards the secondary alteration, which affects only the grey variety of basalt, about 10% of the cryptocrystalline glassy matrix areas of KM-3-74, and about 50% of the matrix areas in

KM-1-74, have interior regions which have altered to a yellowish orange clay (?). The altered zone cuts across and includes relict magnetite grains. Because of the relatively small proportion of glassy matrix in the grey variety (10%), a significant portion of the rock's K₂O can be expected to have been residually concentrated in the glassy phase; if the alteration occurred much later than the eruption, it would have resulted in argon loss and reduced the age measurement. Because the shiny black variety has no alteration of its large amount of glassy matrix (35%), and because there is an indication that the smaller amount of clay-like material in this rock is not an alteration but a secondary filling, we interpret its K/Ar system to be unaltered. To be conservative, its 3.0 Myr age should be regarded as a minimum age because a coexisting variety of the flow, which has endured a similar history, has demonstrably lost argon compared to it.

Magneto-stratigraphy

Studies at East Turkana (formerly Lake Rudolf)⁷ and at Omo⁸ have demonstrated the usefulness of magneto-stratigraphy as a geochronological tool in the terrestrial Plio-Pleistocene sediments of East Africa. One of us (T.J.S.)

been deposited during a time of reversed polarity, with the irregularity resulting from partial remagnetisation during exposure to the present normal polarity. Until we are able to establish the precision of the boundaries by more widespread sampling, the uncertainty in the stratigraphic position of the polarity transitions is assumed to be within 10 m. Figure 1 shows the location of these polarity transitions in sections A and B.

Taken together with the absolute age control, provided primarily by the 2.6-Myr BKT₂ tuff and secondarily by the 3.0-Myr basalt age, the dominantly normal palaeomagnetic stratigraphy of the Hadar Formation is interpreted to be of Gauss Epoch age. The two reversed sequences would represent the well-documented Mammoth and Kaena Events which took place about 3.0–3.1 Myr ago and 2.8–2.9 Myr ago, respectively, as determined by numerous geochronologic studies elsewhere in the world^{9–11}. This correlation with the world-wide reversal scale is shown in Fig. 1.

The palaeomagnetic results of the 30 samples collected the previous field season from three parts of the Hadar Formation¹ are perfectly in accord with the new detailed stratigraphy presented here. One problem arises in correlating partial section B of the Ounda Hadar area with

Table 2 Fission track dating results for BKT₂ (tuff)

Sample	No. of zircons	Spontaneous tracks counted	Ps (cm ⁻²)	Induced tracks counted	Pi (cm ⁻²)	Neutron flux (n cm ⁻²)	Uranium content p.p.m.	Age (Myr)*
38–75–1 + 140 mesh	11	140	2.68×10^5	1,495	5.715×10^6	9×10^{14}	190	2.58 ± 0.23

* Mean $\pm$ 1 s.d.

has started a detailed study of the palaeomagnetism of the Hadar Formation. The preliminary results suggest that a magnetic stratigraphy is retained which can be correlated with the palaeomagnetic polarity reversal time scale. So far only one complete section of the formation has been taken, sampling approximately every half metre. This section (section A, Fig. 1) is in the Sidi Hakoma and Kada Hadar drainage, where most of the fossil localities of the Hadar site occur. In addition, a partial section (section B, Fig. 1) with irregularly spaced samples from 1–4 m apart has also been measured in the Ounda Hadar drainage, including the basalt flow. A total of 400 samples were collected.

All the samples were subjected to progressive alternating field (A.F.) demagnetisation in order to determine the direction and stability of their remnant magnetism. The details of the magnetic behaviour of individual samples will be presented after more sections are completed and only a summary is reported here. The major result is that after 200 Oe of A.F. demagnetisation, the remnant magnetism of individual samples falls into three sequences of very uniform normal polarity, and two sequences of predominantly reversed samples with intermixed samples of random polarity. Every tenth sample of section A (about every 5 m) was subjected to further progressive demagnetisation up to 1,000 Oe. All of these further demagnetised samples in the three normal zones maintained their persistent normal polarity with directions close to north. The randomly interspersed intermediate samples from the dominantly reversed sequences which were defined at 200 Oe of demagnetisation, generally revealed a reversed or more nearly reversed remnant direction on further demagnetisation. The reversed samples in these sequences maintained their reversed polarity. Thus, the higher field demagnetisation enhanced the contrast evident between the two kinds of sequences. We interpret the two sequences with dominantly reversed remnant magnetisation to have

Section A, because 40 m of reversed sediment occur below the ostracod marker bed in Section B and only 10 m occur below this marker in Section A. The thickness of the Sidi Hakoma Member in Section B is 90 m compared to only 60 m in Section A. The present magneto-stratigraphy suggests that most of this east to west variation in thickness of the Sidi Hakoma Member can be ascribed to the very upper part of the member. To what extent this thickness variation is due to varying rates of deposition or to unconformities is not known.

If interpretation of the age framework of the Hadar Formation continues to be substantiated, then more detailed palaeomagnetic studies may allow for additional precision in the stratigraphy, palaeogeography and faunal evolution at the Hadar site. At present, the geochronology and palaeomagnetism suggest for the Hadar Formation and its rich fauna a Gauss Epoch age that spanned from somewhat more than 3.1 Myr ago to somewhat less than 2.6 Myr ago.

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letters to nature

The rings of Uranus

At least five rings encircle the planet Uranus—as indicated by five brief occultations of the star SAO 158687 that occurred both before and after its occultation by Uranus on 10 March 1977. We observed these events with our three-channel occultation photometer¹, attached to the 91-cm telescope aboard the Kuiper Airborne Observatory (KAO). Both Uranus and the star were contained within a focal plane aperture whose diameter, projected onto the sky, was 46 arc s. A beamsplitter and focal plane television system allowed us to monitor the position of the image in this aperture simultaneously with the photometric measurements. The wavelengths of the three photometric channels used were chosen to yield favourable ratios of starlight to Uranus light² and are given in Table 1. For each channel a cooled photomultiplier (RCA C 31034), connected to a photon-counting system, was used as a detector. The data were recorded as a continuous series of 10-ms integrations of photon counts, and a strip chart recorder displayed the signals from our channels 2 and 3 so that we could monitor the progress of the occultation in real time.

Table 1 Centre wavelengths of filters

Channel no.	Centre wavelength (Å)	Passband (Å)	Star counts* Star+background counts
1	6190	80	0.15
2	7280	200	0.32
3	8520	210	0.42

*These ratios were obtained from the observed signal levels.

The final predictions^{3,4} for the occultation indicated that the northern limit of the occultation would pass south of northern Australia, but its precise location was uncertain. Accordingly, the course of the airborne observatory was planned to be as far south as possible. At about 20 h 00 min UT, the KAO assumed a course that allowed tracking on Uranus, and continuous data recording began at 20 h 05 min 40 s UT. A sharp decrease in the recorded light intensity occurred at 20 h 11 min 43 s UT and several seconds elapsed before the signal returned to its original level. This event was followed by several others of shorter duration within the next few minutes. The cause of these events was initially thought to be momentary failures of the telescope tracking system, but careful examination of the Uranus image on the television monitor showed the tracking to be steady. Another cause considered was thin cloud, but at our altitude of 41,000 feet this was unlikely. The infrared water vapour monitor on board the KAO confirmed that no clouds were present. We concluded that the drops in intensity were definitely light lost from the star, since the dips had the proper ratios at different wavelengths (see Table 1). For this case the only plausible cause of these brief intensity dips would be occultations by small bodies, probably associated with Uranus.

About 30 min later, Uranus occulted the star. The occultation lasted about 25 min and had a midtime of 21 h 06 min UT, in good agreement with the predictions based on the

position of the star and Uranus determined by Franz and Wasserman⁵. We then continued our observations in expectation of recording more brief occultations. Five such events were recorded before the data recording was stopped at 22 h 17 min 40 s UT, due to the high level of morning twilight. Similar brief occultations of SAO 158687 when Uranus was near it were also observed by Millis, Birch and Trout at Perth^{6,7}; by Bhattacharyya and Kuppaswamy at Kavalur^{8,9} and by Churms⁶ at Cape Town.

Table 2 contains the midtimes and approximate durations of the brief occultations that we observed. The approximate location of the Airborne Observatory was lat. 50°20'S, long. 82°11'E for the pre-immersion events and lat. 50°21'S, long. 101°19'E for the post-emersion events. In Fig. 1 we have plotted our light-curves (channel 2) for both sets of events after averaging the data over intervals of 0.5 s. The intensity parameter $\phi(t)$ equals 1.0 for the unocculted intensity of SAO 158687 and 0.0 when the star is totally occulted. We have not attempted to remove from these data possible long-term drifts in sky background light and instrumental gain, which could amount to a few per cent. The slow rise in signal level starting at 21 h 52 min UT is caused by morning twilight.

We assumed that the bodies causing the occultations lie in the satellite orbit plane of Uranus and computed their distance from the centre of Uranus. The angle between the line of sight and the normal to the satellite orbit plane was 36.6°. In this computation, the radius of Uranus was set equal to 25,900 km (ref. 8). Then the chord length observed when Uranus occulted the star³ was used to fix the position of Uranus relative to the star. The motion of the KAO was not taken into account in this calculation, however, thus introducing systematic errors of several hundred km, which can ultimately be eliminated. But, since the groups of events occurred at approximately equal times before and after the Uranus occultation itself, the errors in the relative distances between the immersion and emersion events should be less than 100 km.

Table 2 Short occultation events

Pre-immersion events		Post-emersion events	
Midtime (UT)	Approximate duration (s)	Midtime (UT)	Approximate duration (s)
h min s		h min s	
20:11:46	7	21:45:52	1
20:16:03	1	21:47:06	1
20:16:58	1	21:49:45	1
20:19:34	1	21:50:39	1
20:20:55	1	21:54:06	3

The striking impression from Fig. 1 is that the occultations in both groups of events occur at corresponding distances from Uranus. Moreover, the amplitudes and durations of the corresponding events are similar. We feel this is strong evidence that the occultations were caused by five narrow rings that encircle the planet, rather than random occultations caused by small satellites within a broad belt as we and the Lowell group had originally suggested^{3,9}. We have tentatively labelled the rings with the letters α - ϵ , beginning with the ring closest to Uranus.

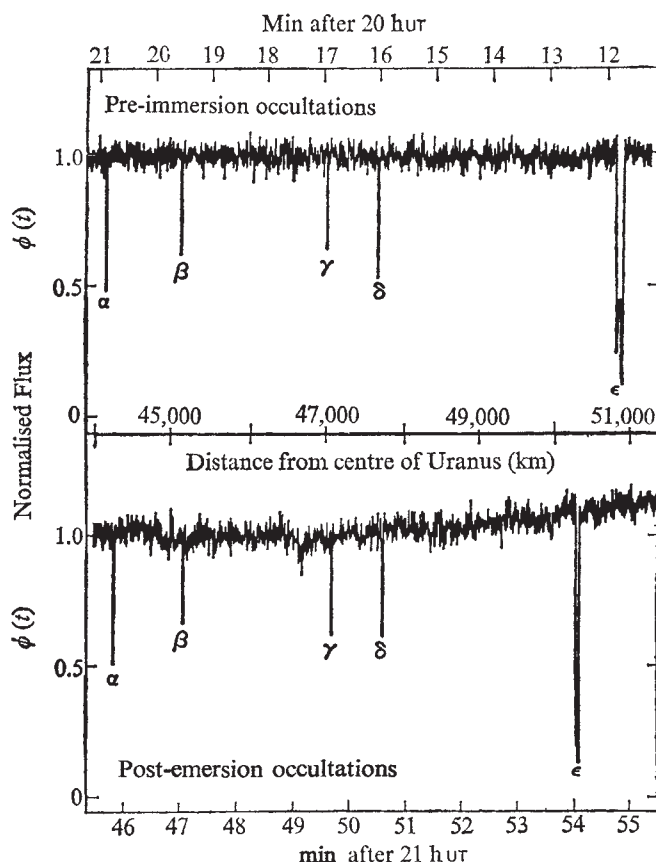


Fig. 1 Light curves (channel 2) showing the five brief occultations observed both before and after the occultation of SAO 158687 by Uranus on 10 March 1977. The intensity scale $\phi(t)$, has been normalised to the unocculted intensity of SAO 158687, and on this scale the amount of starlight occulted ranges from about 0.4 (β and γ events) to about 0.9 (ϵ events) of the total. An approximate distance scale has been computed under the assumption that the bodies causing the occultations lie in the orbit plane of the Uranus satellites. The equal number and symmetry of the occultations suggest that four narrow rings (~ 10 km wide) and a broader ring of variable thickness (ring ϵ , 50–100 km) encircle Uranus. Beginning at 21 h 52 min UT, the start of twilight caused a slow rise in the signal level.

Since the KAO data was the only set reported to contain both the pre-immersion and post-emersion occultation events, we were in a unique position to infer the presence of narrow rings. Subsequent calculations by Marsden⁹ showed that the times of the events observed at Perth and Cape Town led to distances from Uranus, within its satellite orbit plane, that closely agreed with those obtained from our data. Hence the interpretation that these events were caused by narrow rings encircling Uranus has already survived a stringent observational test. (We note a ~ 800 km discrepancy between Marsden's distance scale and our own that arises from different assumptions for our respective calculations. But, the distances of corresponding pre-immersion and post-emersion events are nearly equal for either calculation.)

Our light curves also contain other brief occultations, significantly shallower than the ring α – ϵ events, and are probably caused by additional material within the ring system of Uranus. One example is the decrease in signal that occurs shortly before the ring γ occultation on emersion (see Fig. 1), and there is some indication in our data of similar occultations that correspond to the latest two events reported by Millis, Wasserman and Birch⁷. Our figure does not include this time interval. As yet we have not searched our data for long, shallow occultation features that would correspond to broad rings of low optical depth.

Ring ϵ seems markedly different from the other four in

several respects. It causes much deeper occultations of the star that last longer, but are of unequal duration (Table 2) for the two sections of ring ϵ probed by our observations. Also the times of the occultations by ring ϵ do not agree with its being circular and in the plane of the satellite orbits.

Possibly ring ϵ is elliptical, or has an orbit plane inclined to that of the satellites; it may be that what we have identified as a single ring is actually two incomplete rings. From our data alone, we cannot confirm or rule out any of these explanations, and as yet cannot suggest a more sophisticated model to explain the times and durations of the occultations caused by this ring.

To estimate the width of the rings, we note that the Uranus shadow velocity was 12 km s^{-1} and use the occultation durations given in Table 2. These values yield a width of ~ 12 km, projected onto the plane perpendicular to our line of sight, for rings $\alpha, \beta, \gamma, \delta$; ~ 85 km for the immersion section of ring ϵ ; and ~ 35 km for the emersion section of ring ϵ . We emphasise that these widths are only approximate, since we did not include (1) the convolution broadening of the observed occultation durations caused by the projected diameter (~ 6 km; see ref. 10) of the star at Uranus; (2) diffraction effects (Fresnel scale, $\sqrt{\lambda R} \sim 1.5$ km); (3) the projection angle between the apparent stellar velocity and the orientation of the ring; and (4) effects caused by the orbital motion of the rings, with a velocity of 11 km s^{-1} , that would be important if the optical depth of the rings is not uniform on scales comparable to, and larger than, the projected stellar diameter.

Since none of the rings caused a total occultation of the star, we think they are composed of numerous bodies, with sizes of < 6 km (the projected diameter of the star), rather than larger objects. There are no obvious differences in the structure of the occultations at the different wavelengths that we observed. Such differences would be indicative of diffraction effects and/or wavelength dependent extinction and perhaps provide an important clue to the nature of the occulting material. We plan to pursue this analysis further.

Valuable observations of future occultations by the rings can be obtained for stars significantly fainter than SAO 158687 ($m_v \sim +9.5$; see ref. 10); the precise limiting magnitude will depend on the spectral type of the star and the aperture of the available telescope². Since these occultations need not involve an occultation by Uranus itself, we suggest that present lists of appulses involving Uranus be reviewed in search of those that could result in occultations by the rings. Also, future occultation predictions for Jupiter, Uranus, and Neptune should include information about the apparent path of the star relative to the satellite orbit planes of these planets. The possibility that Jupiter has rings comprised of 'rocky' bodies cannot at present be excluded¹¹. Saturn's rings may contain narrow components, of much higher optical depth than the average, akin to the rings of Uranus. An opportunity to check this possibility, but with coarser spatial resolution than the present measurements for Uranus, occurs in October 1977 and January 1978 when the satellite Iapetus is eclipsed by Saturn and its rings¹².

Possibly the Uranus ring system can be detected through direct photography. If the rings have the same albedo as the cloud tops of Uranus, their combined integrated intensity should be $\sim 0.7\%$ of that of the Uranus disk. This ratio should be substantially more favourable for wavelengths within the methane absorption bands².

The distances between the various rings pose an interesting theoretical problem that can possibly be explained by resonances with orbits of the known satellites and will perhaps require the inference of another satellite. All five rings are well within the Roche limit, which is about 70,000 km for Uranus.

The fundamental question of why rings were unique to

Saturn has been posed many times in the past¹¹, and the particular case of why Uranus was thought to have no rings has been addressed also¹². We now ask, why are the rings of Uranus so narrow—in contrast to the broad rings that surround Saturn? The possibility of 'very small bodies, similar to the asteroids' being found between the orbit of the inner-most satellite Miranda and Uranus itself has been mentioned by Alfvén and Arrhenius¹⁴; the narrow rings may well be a manifestation of the 'jet streams', discussed at length by the same authors¹⁵. The theoretical basis for jet streams has not won general acceptance¹⁶, however, and we expect that alternative theories for the narrow rings will become available soon.

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Note added in proof: W. B. Hubbard has reported that three ring occultations were observed by B. Zellner at Perth Observatory.¹⁷

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Detection of rings around Uranus

AN occultation of the ninth-magnitude star SAO 158687 by Uranus on 10 March 1977 was predicted by Gordon Taylor¹ in 1973. Although the original prediction indicated that the occultation would be widely visible from land masses surrounding the Indian Ocean, subsequent work by Franz and Wasserman (at Lowell Observatory) showed that ground-based observations would be possible only from southern Africa and possibly from southwestern Australia². In view of the wealth of new information about Uranus which could be derived from photometric observations of this event³, we decided to attempt observations using the 61-cm Planetary Patrol telescope at Perth Observatory near Bickley, Western Australia. In fact, an occultation of the star by Uranus itself did not occur at Perth. Partial occultations of SAO 158687 by a series of rings around the planet were observed and are described here.

Measurements were made using a conventional single-channel photometer equipped with an RCA C31034B photomultiplier. The observations were made through an

interference filter centred at 8524 Å with a width of 212 Å (FWHM). Since this filter coincides with a strong methane band in the spectrum of Uranus, the signal from SAO 158687 relative to that from Uranus was greatly enhanced³. An identical filter was used by Elliot *et al.* in one channel of their multi-colour observations of this occultation⁴. The signal from the photomultiplier was amplified by a Kiethley Model 301 operational amplifier and displayed on a strip-chart recorder. The response time of this system for a full-scale deflection is less than 0.5 s. In addition, the signal from the amplifier was fed to a voltage-to-frequency converter whose output was recorded on magnetic tape. This feature provides for digital output of the data at high time resolution, but its recording capacity is limited to 45 min.

During the observations, Uranus was centred in a 28-arc s diameter aperture. Measurements started at 20 h 00 min 00 s UT and continued well into morning twilight with brief interruptions at 5-min intervals for recentering the planet. It is clear from these observations that an occultation of SAO 158687 by Uranus itself did not occur at Perth. But, between 20 h 10 min and 20 h 24 min—well before the predicted time of the occultation of the star by Uranus and before the digital recording system had been turned on—five brief partial occultations of SAO 158687 were detected with the chart recorder. The midtimes, durations, and depths (total occultation=1.0) of these events are listed in Table 1. We estimate a maximum uncertainty in the listed midtimes of ± 0.2 s.

Table 1 Occultations of SAO 158687

Event	UT h min s	Duration (s)	Depth
1	20 10 33.2	8.2	0.94–0.52
2	20 15 58.7	0.7	0.42
3	20 18 44.1	1.5	0.38
4	20 23 19.0	1.0	0.21
5	20 23 42.4	0.9	0.29

Several similar partial occultations were observed by Elliot *et al.*, observing aboard the Kuiper Airborne Observatory about 2,000 km south-southwest of Perth, by Churms at Capetown and by Bhattacharyya and Kuppaswamy at Kavalur^{5,6}. A cursory comparison of the aircraft data and the Perth observations a few hours after the occultations led to a preliminary conclusion by both groups of investigators that several individual members of a satellite belt around Uranus had been detected⁵. The Cornell investigators subsequently discovered, however, that the occultations which they observed prior to the Uranus occultation were very nearly mirror images in time of those which they observed following the Uranus occultation. It was then concluded that the occulting bodies were five narrow circular rings in the equatorial plane of Uranus^{4,6}. These rings have been designated α , β , γ , δ , and ϵ , starting with the innermost ring. The 61-cm Perth observations support this interpretation. If we predict when occultations by these five rings would have occurred at Perth, we find that event number 1 in Table 1 corresponds to the ϵ ring, event 2 corresponds to the γ ring, and event 3 corresponds to the β ring. We did not observe the occultations by rings α and δ at Perth because at their predicted times of occurrence, the observations had been interrupted for about 10 s to recentre the planet. Events 4 and 5, although shallower than the other occultations, are well above the noise level of our observations. It is not yet clear whether these events are present in the observations of other observers, so the nature of the occulting objects is uncertain in these two cases. If these objects are

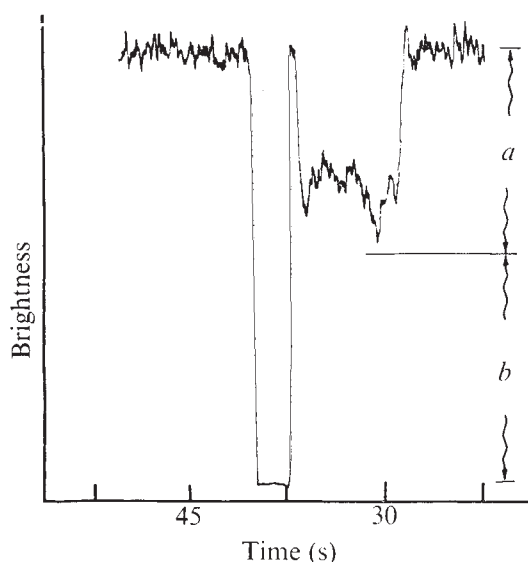
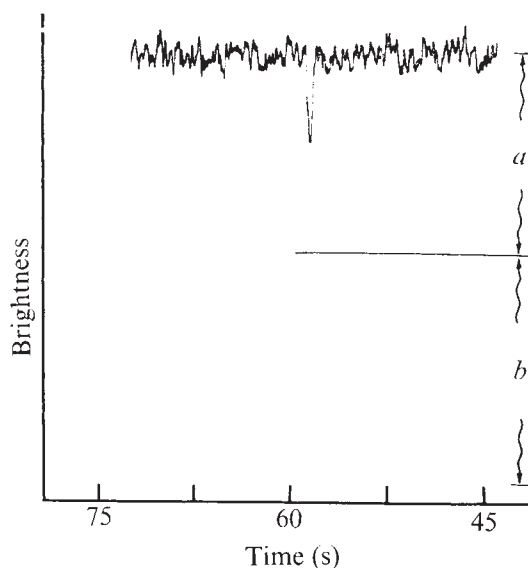


Fig. 1 The occultation light curve observed at Perth for the ϵ ring. The number of seconds past 20 h 10 min 00s is given along the abscissa with time increasing to the left. The sharp drop in signal following the occultation resulted from an interruption of the observations to check the centering of the planet in the photometer's entrance aperture. The contributions to the total signal by SAO 158687 and Uranus are indicated by *a* and *b* respectively.

in circular orbits in the equatorial plane, their orbital radii are approximately 42,000 and 42,250 km.

The Perth light curve of the occultation by the ϵ ring is shown in Fig. 1. This is by far the longest of the five occultations observed at Perth, with the ring extending for a distance of about 90 km along the occultation chord. The abruptness with which the occultation begins and ends indicates that the ring has rather sharp boundaries. Substantial variation in transparency across the ring is seen. Since at no point was the star totally obscured, it can be concluded that the majority of the individual particles in the ring have dimensions smaller than the apparent diameter of the star at Uranus, which we estimate to have been about 4 km. The maximum obscuration which we observed corresponds to an optical thickness of about 2.3,

Fig. 2 The occultation light curve observed at Perth for the γ ring. The number of seconds past 20 h 15 min 00s is given along the abscissa with time increasing to the left. The contributions to the total signal by SAO 158687 and Uranus are indicated by *A* and *B* respectively.



which is substantially larger than that of the B ring of Saturn⁷.

Figure 2 shows the occultation light curve observed at Perth for the γ ring. The very brief duration of this occultation implies that the width of the ring is no more than a few kilometres. The other three occultation light curves observed at Perth are very similar in character to that produced by the γ ring.

Further occultation observations have been reported^{8,9}.

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A new satellite of Uranus

WHILE observing the occultation of the star SAO 158687 by Uranus on 10 March 1977 we came across an unexpected event suggesting the existence of a hitherto unknown satellite of the planet. The observations were carried out at the Kavalur Observatory (lat. 12° 34' 32" N; long 78° 49' 54" E) using a near infrared photometer consisting of a refrigerated EMI 9558B phototube and Kodak Wratten 89B filter attached to the Cassegrain focus of the 102-cm telescope. The individual deflections due to the star and the planet were measured during the previous night, as well as a few hours before the predicted instant of occultation. A 16-arc s diaphragm was used during occultation, whereby the planet and the star could be conveniently contained for several hours around the epoch of occultation. Guiding and monitoring of the objects were done through the 20-cm guide telescope, with periodic checking of the centering in the main telescope. As indicated by the prediction¹, we expected only a grazing occultation to be visible at Kavalur, resulting in a slight diminution in the intensity of starlight owing to the extinction effect by differential refraction in the outer layers of the planet's atmosphere². A very shallow depression of 0.046 magnitudes in the light of SAO 158687 was indeed observed.

About 40 min before the midpoint of this dip we observed at 20 h 19 min 38 s UT a sharp drop in the light curve which lasted 8.9 s (Fig. 1). The phenomenon was visually observed in the guide telescope also, as a sudden disappearance of the star in the field of view followed by an equally sudden reappearance, a few seconds later. Taking the most recent positions of Uranus and that of the star (C. Lecacheux, personal communication), this point is found to be 4".1 away from the centre of the planet at a position angle of 349°. Assuming a circular orbit and correcting for foreshortening due to orientation of the axis of the orbit (assumed to be same as that of the planet), we arrive at a value of 53,000 km for its radius, and an approximate period of 9 h 30 min. The apparent motion of this satellite's shadow with respect to the observer is

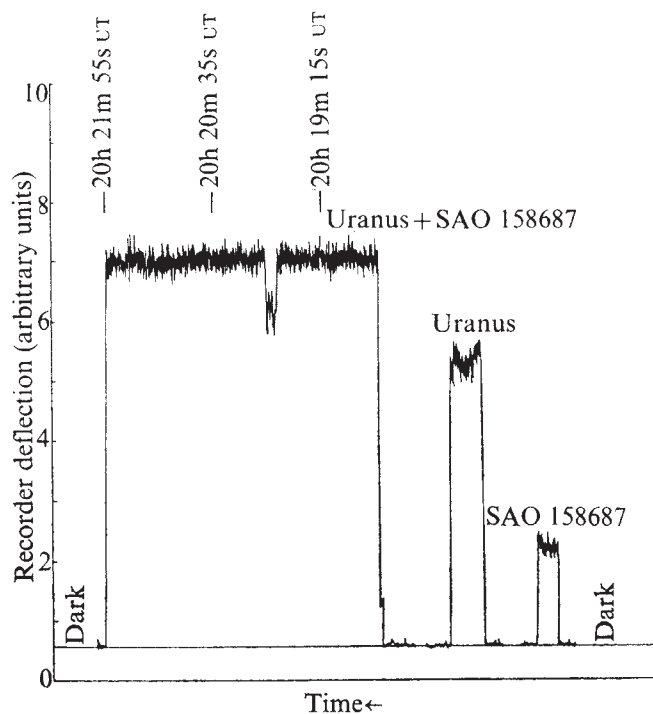


Fig. 1 Light curve obtained during occultation of SAO 158687 by Uranus.

estimated to be 2.74 km s^{-1} , yielding the length of the chord joining the immersion and emersion points as 24 km.

The size of the body can be estimated from the occultation light curve, where the dip is only 53% of the total star light. This can be explained by postulating that the stellar disk subtends an angle comparable to that of the satellite and the occultation in partial. We assume a typical stellar diameter of 3.5×10^{-4} arc s for a K5 star with the appropriate distance modulus³. Then, from a model of the occultation geometry, and taking into account the diffraction near uniform edges, we find that the estimated diameter will be around 70 km. The visual magnitude of the satellite is estimated to be ~ 19 .

We conclude that the body is a new satellite of Uranus.

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Spectral variability in X-ray sources

COE *et al.*¹ have reported that Cyg X-1 and the transient source A 0620-00 both display anticorrelated spectral variability. That is to say, flux enhancements at low energy ($E \lesssim 10 \text{ keV}$) tend to be accompanied by a decline at high energy and vice versa. Both sources may be considered possible black holes; Cyg X-1 is well known, while the candidacy of A 0620-00 ($\equiv$ Nova Monocerotis 1975, distance $\gtrsim 2 \text{ kpc}$)^{2,3} follows if its peak luminosity $\gtrsim 5 \times 10^{38} \text{ erg s}^{-1}$ is identified with the Eddington limit luminosity $1.3 M/M_{\odot} \times 10^{38} \text{ erg s}^{-1}$ for mass accretion. Thus the accreting object has a mass $\gtrsim 4 M_{\odot}$, just greater than the accepted limiting mass for a neutron star. It has therefore been suggested¹ that anticorrelated spectral variability, perhaps resulting from inverse Compton scattering in the accretion

disk^{4,5} may be common to X-ray emitting binary systems which contain black holes. In this paper, we wish to show that anticorrelated variability is not confined to sources which are black hole candidates.

We have examined published spectral data for the above two objects and four other well-observed sources — namely, Cen X-3, Her X-1, Cyg X-3 and Cir X-1. The references to all data used in this paper (more than 50 observations) are too numerous to mention here and are tabulated elsewhere⁶. In order to facilitate comparisons we have selected only those results for which a power law spectrum has been fitted, of the form

$$I(E) = AE^{-\alpha} (\text{keV cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}) \quad (1)$$

In some cases the power law may not have provided the only fit to data but we assume that the quoted spectral index α characterised the spectral shape at the time of observation and allows ready comparison to spectra at other times.

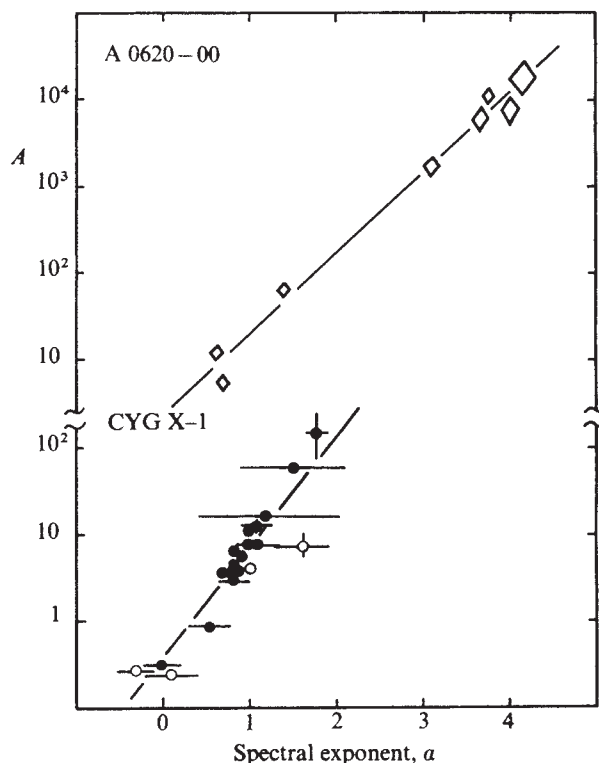


Fig. 1 Spectral variability plots for black hole candidates A 0620-00 (2–15 keV) and Cyg X-1. Open circles: low energy, $\lesssim 30 \text{ keV}$; full circles: high energy, $\gtrsim 30 \text{ keV}$. The lines of best fit are shown.

Spectra displaying anticorrelated variability about a 'pivot' energy E_p are described by $I(E) = A'(E/E_p)^{-\alpha}$ where $A' = \text{constant}$. Comparison with (1) therefore implies that $A'E_p^\alpha = A$, or $\log A = C + S\alpha$, where $S (= \log E_p)$ and C are constant for the source in question. Therefore, in order to better quantify anticorrelated variability we have plotted spectral observations as points on the $(\alpha, \log A)$ plane (hereafter called spectral variability plots, or SVPs), looking for a linear correlation, Fig. 1 shows these data for Cyg X-1 and A 0620-00 and the clearly linear correlations confirm the findings of Coe *et al.*¹. Linear correlation coefficients and the associated negligible probabilities that these correlations could be due to random fluctuations are shown in Table 1. In particular, it is found that $S \sim 1.3$ and 0.9 implying $E_p \sim 20$ and 8 keV for Cyg X-1 and A 0620-00, respectively.

Application of this technique to the other sources (Fig. 2) leads to the surprising result that all four behave in a similar

Table 1 Summary of results

Source	N	$S \pm 2\sigma$	R	P
Cyg X-1	25	1.3 ± 0.2	0.92	< 0.001
A0620-00	8	0.9 ± 0.1	0.99	< 0.001
Cyg X-3	5	1.1 ± 0.3	0.97	0.005
Her X-1	9	1.3 ± 0.3	0.95	< 0.001
Cen X-3	5	0.9 ± 0.5	0.90	0.03
Cir X-1	10	1.3 ± 0.3	0.97	< 0.001

N is the number of observations; R is the correlation coefficient; P is the probability that the correlation arises by chance.

manner to the black hole candidates. Taken alone the result for Cen X-3 is only marginally significant; nevertheless it produces a value for S similar to that for the other sources (see Table 1). In all cases best fit values of S lie between 0.9 and 1.3 corresponding to E_p values between 8 and 20 keV. It would appear therefore that all six sources display anticorrelated spectral variability.

We have discriminated between observations made predominantly below and above 30 keV ('low' and 'high' energy) in order to determine if spectral breaks unrelated to time variability have introduced spurious effects. We have sufficient data for Cyg X-1 to allow formal fits to be made with and without the low energy points. No significant difference in the

quality of the fits or in S values could be obtained. Low and high energy data points are distinguished for all the sources in Figs. 1 and 2 and we conclude that there is no evidence to suggest that the correlation between S and α is caused by energy dependence of the spectral exponent.

This study suggests that anticorrelated spectral variability may occur in X-ray sources other than those favoured as black hole candidates. It will be of interest to see if proposed inverse Compton emission mechanisms which show promise of explaining the observations for the black hole candidates^{4,5} can be generalised for binary systems involving say, neutron stars or white dwarfs.

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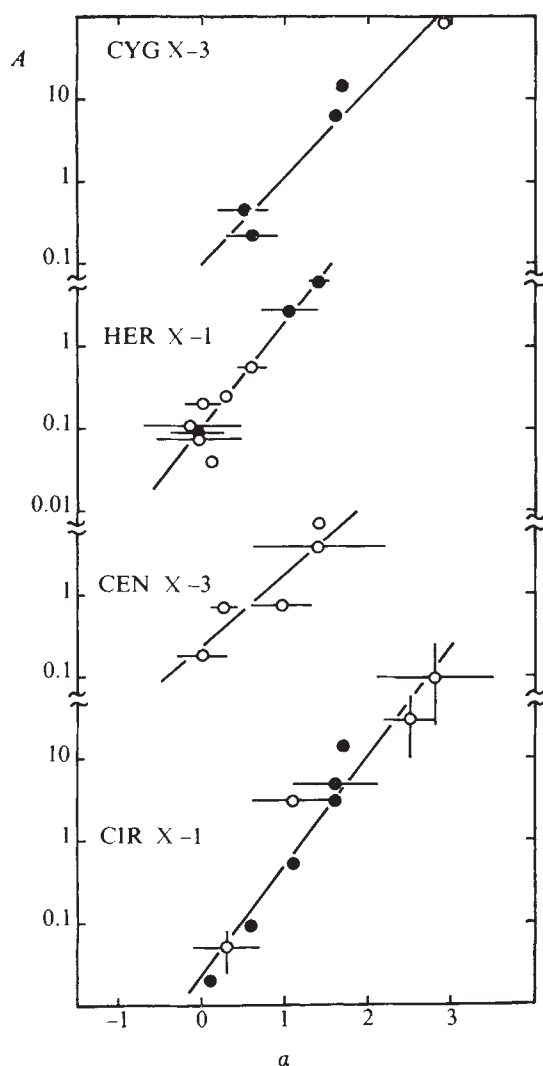


Fig. 2 Spectral variability plots for Cyg X-3; Her X-1; Cen X-3 and Cir X-1. Open and full circles represent low (< 30 keV) and high (> 30 keV) energy observations respectively. The lines of best fit are shown.

Statistical mechanics of microemulsions

At low surfactant concentrations, an oil-water microemulsion may be represented as two immiscible continuum fluids, oil and water, dispersed in one another so as to form two interpenetrating, multiply connected regions, with the surfactant adsorbed at the interface between them. Scriven¹ has pointed out that as the oil/water ratio is increased, three types of structure will evolve in succession: starting from the oil-in-water emulsion to be expected at low oil content, there should be a smooth transition to a bicontinuous structure, in which neither phase can be said to surround the other, followed by a second transition to a water-in-oil emulsion when the oil content is sufficiently high. It seems likely, as Scriven suggests, that the phase diagrams reported for microemulsion systems involve all of these structures. Here we propose a statistical mechanical model for microemulsions, based on a simple procedure for generating random two-fluid geometries. Over certain ranges of the model parameters, the resulting free energy-composition surface shows regions where a bicontinuous phase may coexist with an oil-in-water and a water-in-oil emulsion; such three-phase equilibria have been observed experimentally². Our calculations also suggest a possible explanation of the very low interfacial tensions sometimes found between microemulsion phases.

To determine, at least within a constant of proportionality, the number of configurations available to the oil and water regions in a microemulsion of specified composition, we restrict ourselves to the class of geometries resulting from a two-step process, consisting of a Voronoi tessellation³⁻⁵ followed by random segregation into oil and water domains. N points are first distributed completely at random over the volume V to be occupied by the microemulsion; with each of these Poisson points, we associate the polyhedral region which lies closer to it than to any of the other ($N-1$) points (Fig. 1). In the second step, N_0 of these polyhedra are selected at random to be occupied by oil, and the remainder filled with water; the result is a random two-fluid geometry in which the oil occupies a fraction $v_0 = N_0/N$ of the total volume.

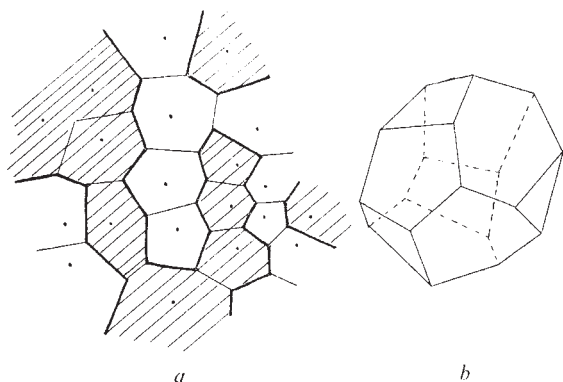


Fig. 1 *a*, Two dimensional representation of the partition process. Shaded regions are occupied by oil, unshaded regions by water; heavy lines indicate the surfactant monolayer. *b*, Typical Voronoi polyhedron.

The number of distinct positions available to a Poisson point is proportional to the volume V , the proportionality constant $1/\omega$ being one of the model parameters. It is the size of ω that determines the scale of the microemulsion structure, and it is through ω that information about the molecular nature of the water and oil fluids enters into the model. The number of configurations for the placement of all N points is then

$$(V/\omega)^N/N!$$

and for each configuration there are

$$N!/N_o!(N-N_o)!$$

ways of assigning the oil and water labels. The configurational entropy of the microemulsion is therefore

$$S = -Vk[\ln(\omega c/e) + v_o \ln v_o + (1-v_o) \ln(1-v_o)], \quad (1)$$

where $c \equiv N/V$ is the concentration of Poisson points.

To translate equation (1) into predictions about the thermodynamic behaviour of the system, we need an expression for the surfactant concentration σ in terms of c and v_o . We assume that all of the surfactant is located in a monolayer at the interface between the oil and water regions, and that there are no oil-water contacts unmediated by such a monolayer. The surfactant concentration thus fixes the oil-water interfacial area.

The total area per unit volume of the surface generated by the Voronoi tessellation³ is $2.91c^{1/3}$, and the total length of the polyhedral edges in unit volume is $5.83c^{2/3}$. Only a fraction $2v_o(1-v_o)$ of the total surface will actually separate oil from water polyhedra; furthermore, since each edge is shared by three polyhedra, the probability that it lies on the oil/water interface is $3 v_o(1-v_o)$.

We assume that a plane interface between the oil and water regions adsorbs α moles of surfactant per unit area, but that a curved surface will, because of crowding of surfactant segments, have a somewhat lower surfactant capacity. This curvature effect is introduced into the model by lowering the amount of adsorbed surfactant by β moles per unit edge length. The expression for σ derived from these considerations is

$$\sigma = (5.82\alpha c^{1/3} - 17.5\beta c^{2/3})v_o(1-v_o) \quad (2)$$

Equations (1) and (2) together may be used to determine the configurational free energy $G = -TS$ as a function of σ and v_o (in calculating c for a given value of σ , we use the lower of the two roots of equation (2)). If the results are expressed in reduced quantities

$$G^* = \omega G / eVkT, \quad \sigma^* = \omega^{2/3} \sigma / 17.5 e^{2/3} \beta \\ \alpha^* = \omega^{1/3} \alpha / 3.0 \beta e^{1/3}$$

the reduced free energy G^* will depend only on σ^* , v_o , and α^* ; the shape of the free energy surface, and therefore the phase equilibria to be expected, are entirely determined by the dimensionless parameter α^* .

Figure 2*a* shows plots of G^* against v_o for various values of σ^* at $\alpha^* = 2.0$; below $\sigma^* = 0.247$, the plots show two symmetrically placed minima, the locations of which represent the compositions of two microemulsion phases in equilibrium. In Fig. 2*b* we have plotted (schematically only, in order to show the region of negative curvature) the minimum values of G^* from Fig. 2*a* against σ^* . The two compositions at which the double tangent touches the curve correspond to a bicontinuous phase ($\sigma^* = 0.247$, $v_o = 0.5$) in equilibrium simultaneously with two other microemulsions of lower surfactant content ($\sigma^* = 0.102$, $v_o = 0.121$, and $\sigma^* = 0.102$, $v_o = 0.879$)—in other words, a three-phase equilibrium.

Figure 3 shows the three- and four-phase domains in the three-component phase diagram for various α^* : a mixture whose overall composition is located in the interior of a triangle or quadrilateral separates into phases with compositions given by the vertices; for α^* values outside the range 1.63–3.5 only two-phase equilibria are possible. The apparent violation of the phase rule by the four-phase regions found below $\alpha^* = 1.75$ results from the symmetry between the oil and water components imposed by our model. The slightest perturbation of this

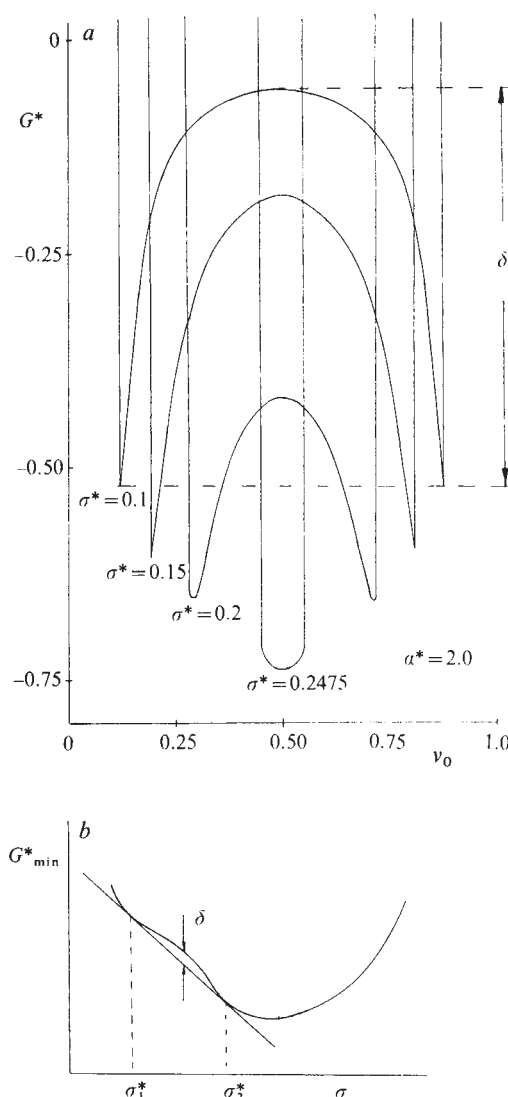


Fig. 2 *a*, Reduced free energy plotted against volume fraction of oil at $\alpha^* = 2$ and several values of σ^* . *b*, Schematic plot of minimum free energy against σ^* .

symmetry leads to replacement of the top and one side of a four-phase quadrilateral by the corresponding diagonal; the resulting triangle represents a three-phase equilibrium.

In addition to predicting, at least qualitatively, the existence of the multiple phase equilibria observed experimentally, our model also suggests the origin of the very low interfacial tensions between the separated microemulsion phases observed for certain systems². In Fig. 2b the maximum difference δ between the G^* curve and the double tangent is 0.0008, to be compared with a difference $\delta' = 0.47$ between the reduced free energies for the corresponding homogeneous and phase-separated mixtures in Fig. 2a. Although no quantitative calculations have yet been made, the implication is strong that a very thin layer of the bicontinuous phase can reduce the interfacial tension between a water-in-oil and an oil-in-water microemulsion by several orders of magnitude.

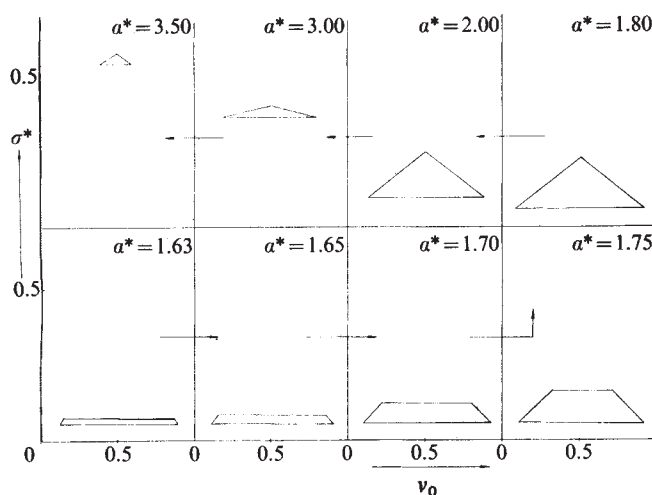


Fig. 3 The three- and four-phase regions at various values of α^* .

Our aim has been to offer a minimal model of microemulsion phase equilibria, a model which leads to a purely entropic free energy function. In the absence of composition-dependent energy contributions, all phase equilibria should be independent of temperature, in apparent contradiction to experimental fact. It should be remembered, however, that the model assumes an ideal surfactant, one which exists only at the water/oil interface. The balance between hydrophilic and lipophilic interactions required for such optimal performance will in general be quite sensitive to temperature changes, particularly in the case of the surfactant-cosurfactant systems used in practice.

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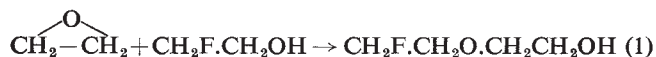
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Toxic fluorinated compounds as by-products of certain BF_3 -catalysed industrial processes

We have embarked on a programme of toxicity tests on a range of industrial chemicals that are manufactured using boron trifluoride (BF_3) as a catalyst. Although this study is still in its early stages, we feel it opportune to draw attention to the fact that in some of the samples we have detected low concentrations of fluorinated compounds, some of which merit serious toxicological concern. The most notable example we have uncovered concerns some industrial chemicals that are manufactured from ethylene oxide using BF_3 as catalyst. On the basis of the results of toxicological studies of these products, we suspected fluoroacetate precursors as contaminants, and subsequent chemical analysis has confirmed the presence of low concentrations of 2-fluoroethanol and/or 2-(2-fluoroethoxy) ethanol. These two compounds are known to be very toxic to mice (LD_{50} 10 and 15 mg kg^{-1} respectively) and are regarded as fluoroacetate precursors^{1,2}. The latter is the addition product of 2-fluoroethanol and ethylene oxide (equation 1), and 2-fluoroethanol is the formal addition product of fluoride ion to ethylene oxide (equation 2).



This explanation of the genesis of the two fluoroacetate precursors points, not to BF_3 itself, but to fluoride ion as the source of the toxicants. That F^- is a common by-product of BF_3 -catalysed reactions is further supported by the identification of tetrafluoroborate ion (BF_4^-) as a contaminant of some of the other samples we have examined. For this chemical is the readily-formed product of interaction between F^- and BF_3 . The origin of fluoride ion is unclear; it might derive by solvolysis of BF_3 —or of silicon tetrafluoride, SiF_4 , a known contaminant of commercial BF_3 .

Fuller details of these studies will be published elsewhere.

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Aragonite in Permian Reefs

PRESERVATION of aragonite in fossils decreases the further we go back in the geologic record. In the Palaeozoic, aragonitic remains have been found only in clayey-bituminous deposits which have largely inhibited diagenetic alterations of unstable mineral phases (for example, Buckhorn Asphalt and Kendrick Shale, both Upper Carboniferous of USA¹, and Upper Oil Shale Group, Lower Carboniferous of Scotland²). Palaeontological information preserved in these facies is restricted, however, to those organic remains which lived in the equivalent basinal environments or were transported into them. Original mineralogy and microstructures of ancient reef organisms can generally only be deduced from analogies with equivalent younger counterparts. The oldest known reef-organisms with preserved aragonitic microstructures were

Table 1 Microstructure, cement and aragonite contents in typical Permian reef organisms of Djebel Tebaga (southern Tunisia)

Specimen no.	Determination	Microstructure of the skeleton	Cement	% of aragonite
T 31/6	Solenoporacean alga	Recrystallised	Blocky calcite	37
T 31/7	<i>Stellispongia bacilla</i> Termier & Termier	Spherulitic	Blocky calcite	11
T 20/6	<i>Stellispongia</i> cf. <i>lobata</i> (Parona)	Recrystallised	Locally acicular aragonite, mostly blocky calcite	20
T 31/8	<i>Stellispongia</i> sp.	Irregular	Blocky calcite	20
T 7/1	<i>Disjectopora</i> sp.	Spherulitic (?)	Acicular aragonite + blocky calcite	19
T 26/1	Chaetetid sclerosponge	Spherulitic	Blocky calcite	33
T 209	Chaetetid sclerosponge	Spherulitic	Blocky calcite	21
T 7/2	<i>Plerophyllum</i> (<i>Plerophyllum</i>) sp.	Recrystallised	Blocky calcite	—
T 31/10	<i>Permosoma permotessellata</i> (Parona)	Clinogonal	Blocky calcite	6.5

found in the Upper Triassic (Lower Carnian) of the Southern Alps³. Here, early burial of patch reefs and reef detritus by clayey-tuffaceous sediments and porosity diminution of the carbonates by subsequent cementation have probably inhibited transformation of aragonitic microstructures into calcite⁴. In this report we describe investigations of microstructures and diagenesis in calcareous sponges which have revealed aragonitic primary structures and cements in material from the Upper Permian of Djebel Tebaga, Southern Tunisia.

The Upper Permian specimens were collected from small patch reefs and their marginal reef detritus, intercalated in an irregular sequence of sandstones and marls, crinoidal, brachiopod and fusulinid limestones⁵. Calci-sponges (Inozoa, Sphinctozoa) and sclerosponges are the main reef-builders. They are associated, especially in the marginal reef areas, with stromatolites and solenoporacean algae. The enigmatic tabulate coral-like fossil *Permosoma permotessellata* (Parona) is, in places, a successful frame-builder. Rugose corals, so close to their presumed extinction

on the Permian-Triassic boundary, have been found only in two places and do not contribute to reef construction. The marginal areas of the patch reefs and the surrounding reef detritus have yielded the greatest number of individuals and species. Externally the specimens are rather well preserved, beautifully washed out by recent erosion, but appear highly altered by diagenesis. Calcareous sponges are often partly or totally dolomitized.



Fig. 1 *Disjectopora* sp., Upper Permian, Djebel Tebaga (Tunisia). T 7/1. Vertical thin section through astrorhizal canal with two tabulae, showing spherulitic aragonite cement along the outer margin and blocky calcite in the centre of the voids. Primary microstructure of the skeleton obliterated by recrystallisation. Bar represents 100 μ m.

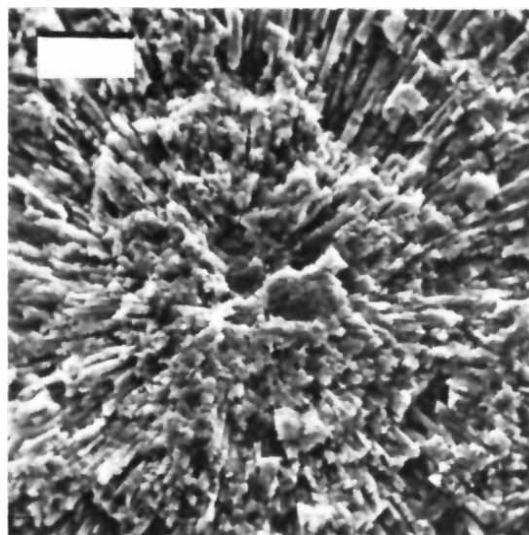


Fig. 2 Chaetetid sclerosponge, Upper Permian, Djebel Tebaga (Tunisia). T 209. Spherulitic microstructure of the skeleton, partly recrystallised. Bar represents 10 μ m.

The occurrence and distribution of aragonite was first shown by staining thin sections with Feigl solution. Thirty specimens, mostly calcareous sponges (pharetronids, sclerosponges), a few corals, calcareous algae (solenoporaceans) and *Permosoma* were X-rayed for determination of calcite, aragonite and dolomite concentrations. In 20 samples aragonite contents were between 1% and 37%; 10 samples were free of aragonite, and in three samples dolomite contents were up to 36%. Aragonite is patchily distributed in the massive skeletons of the above groups and/or as a thin veneer on the skeletal elements. This veneer is the first generation of cement, with acicular shaped crystals which were often translucent (Fig. 1). The preservation of organic microstructures does not necessarily improve with an increase in aragonite content. As can be seen from Table 1, even with complete recrystallisation of the original crystal fabric, the remaining aragonite content may reach 20%. As has already been stated for Triassic calcareous sponges³, the occurrence of certain microstructures is not a consistent generic feature of calcareous sponges. Representatives of *Stellispongia*, for instance, which exhibit

clinogonal or irregular arrangements of aragonite crystals in the Triassic, have spherulitic microstructures in Permian species: the latter fabric is the most common in Permian calcareous sponges, having been encountered in inozoans⁶, sphinctozoans⁷, sclerosponges (Fig. 2) and stromatoporoids (disjectoporids). Clinogonal microstructure has been found in one specimen of *Stellispongia*, and the tube-walls of *Permosoma permotessellata* (Parona). Tabulae and opercula of this species show irregularly spherulitic structure⁶.

The surprising preservation of aragonitic organic microstructures and cements in the Permian reef deposits of Tunisia is being subjected to further mineralogical and geochemical investigations. Material so far examined demonstrates that even 'hopeless' facies with obviously complete diagenetic alteration may still retain a large amount of mineralogical and microstructural information.

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The Grenville Province as a shear zone

PALAEOMAGNETIC and structural data on the Grenville Province in North America are best explained if the Grenvillian 'orogeny' was caused by right-lateral simple shear of the entire Province. Relative displacement of the edges of the belt is estimated at 200–300 km.

Apparent polar wander paths (APW) for the Grenville Province are clearly distinct from the North American path at the corresponding time (Fig. 1). This abnormal distribution has been explained by a collision between the Province and the rest of the continent^{1–3} or by an extra loop of the North American APW path⁴. Relative ages of magnetisation from minerals that have given two distinct polar positions would all seem to indicate however that the collision model is palaeomagnetically more satisfactory. Geological evidence, on the other hand, militates against the existence of a suture anywhere in the Grenville Province. The model thus requires that the postulated suture be hidden under the Appalachians, and that the Grenville Province be an ancient analogue of the modern Tibetan plateau^{1,5}.

Another hypothesis that satisfies palaeomagnetic constraints is that the Grenville 'track' formed by counter-clockwise rotation of individual structural elements inside the Grenville Province. Samples that show two directions of magnetisation separated by very large angular distances come from severely deformed areas, whereas individual poles closest to the normal position for North American poles at that time come either from rocks close to the Grenville Front or from highly resistant, moderately deformed zones. By contrast, poles from samples collected in strongly deformed belts, or far from the Grenville Front plot farther away along the Grenville track (Fig. 1). The length of this track suggests that samples from intensely deformed rocks may have suffered up to 60° of counter-clockwise rotation relative to other North American poles.

This interpretation of palaeomagnetic data makes it worth re-examining the structure of the Grenville Province. A

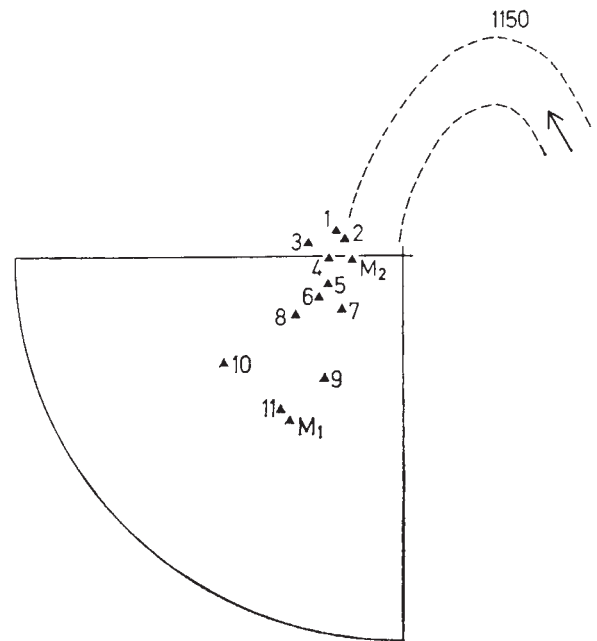


Fig. 1 Stereographic projection of palaeomagnetic pole positions from the Grenville Province. Michael gabbro (1); Grenville Front anorthosite (2); Shabogamo gabbro (3); Unfraville gabbro (4); Lac St Jean anorthosite (5); St Urbain anorthosite (6); post-Grenville Frontenac dikes (7); Wilberforce pyroxenite (8); Ottawa diorites (9); Magnetawan gneisses (10); Lac Allard anorthosite (11); M₁ and M₂, two pole positions from the Morin anorthosite. The apparent polar wander path for North America between 1150 and 950 Myr BP is sketched in.

northeasterly trending shear zone closely follows the Grenville Front⁶ and another, less developed, parallel belt marks the southeastern margin of the Province (Fig. 2). Between these two shear zones, the Province is broken into a number of blocks separated by north-northeasterly trending shear belts—'straight belts'⁷ such as the Chibougamau–Gatineau lineament⁸—that, in numerous cases, are underlined by mylonites. This pattern, visible in Fig. 2, is also present at larger scales.

Although strain measurements in the field show predominant flattening and/or constriction, deformation in straight belts must occur at least in part by simple shear at the scale of the belt. Rigid blocks of small dimensions are represented by granitic plutons and anorthositic massifs, whereas larger ones seem to correspond to fragments of an older basement that have escaped the brunt of Grenvillian deformation. Shear belts tend to follow strips of metasediments (marble and paragneiss of the Grenville Group) but also appear to have remobilised the basement where its pre-existing structures were suitably oriented. Where the map pattern indicates relative displacements along the edges of blocks, they correspond to counter-clockwise rotation of the blocks. Their rotation is thus antithetic to that of the Grenville belt as a whole. The structural pattern is thus everywhere compatible with deformation of the Grenville Province by overall simple shear (Fig. 2).

According to geochronological data, the Grenvillian event did not start much before 1150 My ago and was over by about 950 My ago. During this interval, the entire region was heated sufficiently for all K–Ar clocks to be reset. Rb–Sr isochron dates have also been reset in the Chibougamau–Gatineau shear belt, but this was not the case in the more rigid block located to the west of it.

By analogy with Labrador, anorthositic suites are commonly thought to be about 1400 Myr to 1500 Myr old, but Rb–Sr and U–Pb dates on the Adirondacks⁹, Morin¹⁰ and Lac St Jean complexes¹¹ are Grenvillian. Low ⁸⁷Sr/⁸⁶Sr initial ratios and apparent syntectonic emplacement of the

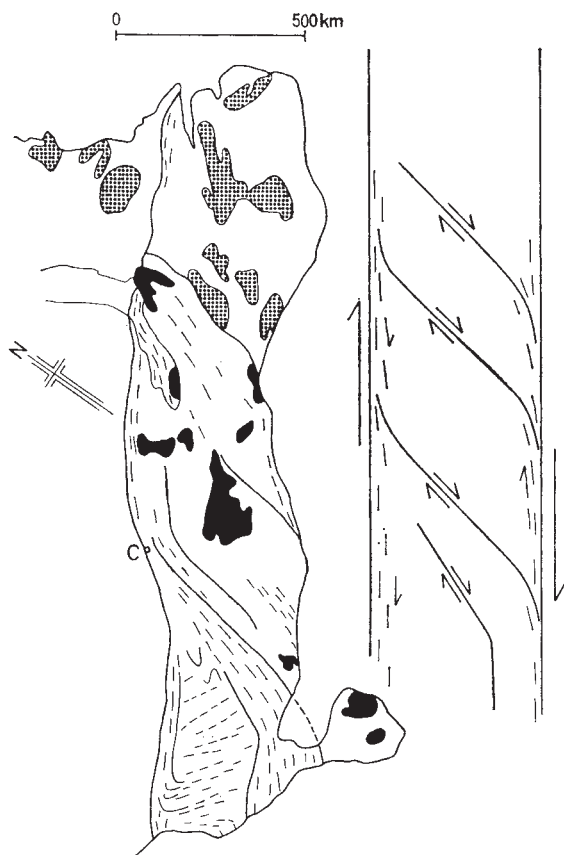


Fig. 2 Sketch of the Grenville Province and suggested shearing mechanism. Thicker continuous lines indicate boundaries of subprovinces; thin dashed lines show structural trends. Anorthosite massifs with mafic roots are dotted in, those without mafic roots are black. C, Chibougamau on the Grenville Front, at the north end of the Chibougamau-Gatineau lineament.

Morin pluton¹² indicate that those three anorthositic complexes are coeval with the Grenvillian deformation. In fact, the peculiar shape of the Morin massif may result from clockwise rotation of this pluton during its emplacement. The lack of a mafic root in all three massifs, by contrast with those of eastern Labrador, probably reflects syntectonic emplacement.

Numerous field studies have demonstrated the synchronicity of deformation and metamorphism in the Grenville Province, so that some heat source must have been available during deformation. Without this excess heat, it is indeed doubtful that such a wide zone (~300 km) would have been deformed by simple shear. The 1500 Myr old anorthositic suite occurs along a small circle that is in close geographical proximity to the Grenville Province. It is possible that the same deep-seated hot belt responsible for these plutons may have underlain the Grenville Province around 1,100 Myr to 1,000 Myr ago. As the Province follows a small circle of about 60° radius, one may envisage its deformation to have corresponded to clockwise rotation relative to North America around an Eulerian pole located at the centre of the circle. In more general terms, this style of deformation appears to be characteristic of Proterozoic mobile belts including the Pan-African ones¹³⁻¹⁵.

From apparent displacement of possibly equivalent rocks, the sum of right-lateral strike-slip motion across the Province is about 200–300 km, a figure comparable to those derived from apparent rotation of palaeomagnetic poles.

In this interpretation, the Grenville Front can be at various points a fault, a thrust or a metamorphic boundary, depending upon the location and the orientation of its

various segments relative to the overall shear direction. The synchronous opening of the Keweenaw graben is taken to represent a fracturing of the old brittle Archean craton, caused by right-lateral shear in the Grenville Province, whereas the intrusion of the Mackenzie diabase dyke swarm represents the tension phase that preceded and accompanied deformation in the Province¹⁶.

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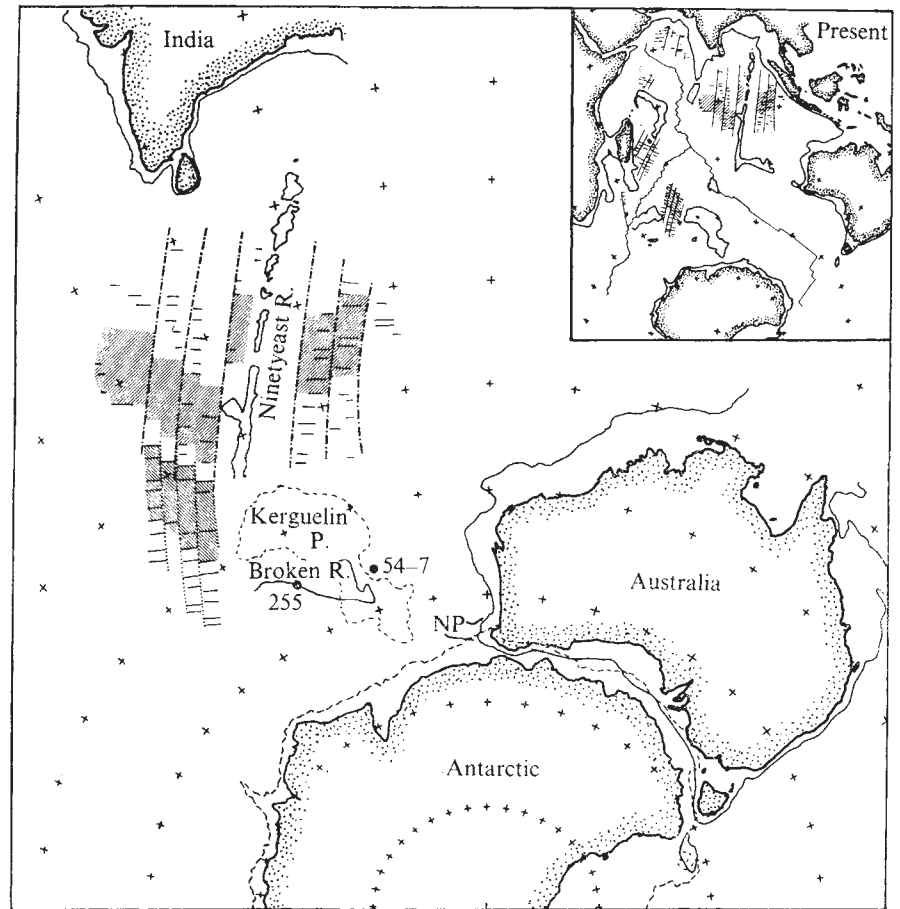
Implications of a revised fit between Australia and Antarctica for the evolution of the Eastern Indian Ocean

GRIFFITHS¹ suggested that the computer fits of Australia and Antarctica²⁻⁴ are not supported by geology and that a better fit can be obtained by moving Australia 250 km westwards along the coast of Antarctica in the computer fitted reconstructions. Griffiths's revised fit eliminates the overlap of the South Tasman Rise, now known to be of continental origin⁵, and the Iselin plateau. Laird *et al.*⁶ showed that new geological evidence from Tasmania and East Antarctica favour such a revised reconstruction over the older computer fits. We present a slight modification to the fits given by Griffiths and by Laird *et al.* that, in addition, allows a good fit of magnetic anomaly lineations and fracture zone trends on the India⁷ and Antarctic⁸ plates (Fig. 1). This revised reconstruction thus implies that India and Australia have been parts of the same plate since the time of anomaly 22 (about 50 Myr ago). It also substantially reduces the size of the postulated Greater India⁹⁻¹¹ (Fig. 2).

Figure 1 shows this reconstruction of the eastern Indian Ocean at anomaly 22 time. Australia is rotated to Antarctica by 30.9° about a pole at 11.9°N, 30.8°E. India is attached to Australia and rotated with it in Fig. 1. Magnetic lineations and fracture zones^{7,8,12} are also shown, with anomalies 23 to 28 shaded. The fit was done by eye, using the geological data⁶ as a constraint and the 2,000-m isobaths as the continental edges. There is some overlap in West Australia (also present in the computer fits²⁻⁴), and a gap in the Australian Bight, but the fit in the eastern sector is good.

As in the computer reconstruction of McKenzie and Sclater⁴, there is a large overlap of Kerguelen Plateau and Broken Ridge. Calcareous sediments of Cretaceous age have been recovered from both these features^{13,14} which indicates that they were elevated structures before Australia and Antarctica separated. Broken Ridge is an elongate tilt-block structure¹⁵ with a steep southern slope dropping into the Diamantina fracture zone. Kerguelen Plateau has been poorly mapped, but in outline it does seem to divide along approximately 55°S into northern and southern sections.

Fig. 1 Reconstruction of eastern Indian Ocean at anomaly 22 time. Antarctica fixed. Isobaths are 2,000 m for Australia and Antarctica (dashed), 3,000 m for Kerguelen Plateau (dashed), Broken Ridge and Ninetyeast Ridge north of inferred position of anomaly 22 (ref. 7). DSDP site 255 on Broken Ridge¹³ and the site of Eltanin core 54-7 (ref. 14) are shown. NP, Naturaliste Plateau. Equal area projection, 10° grid spacing.

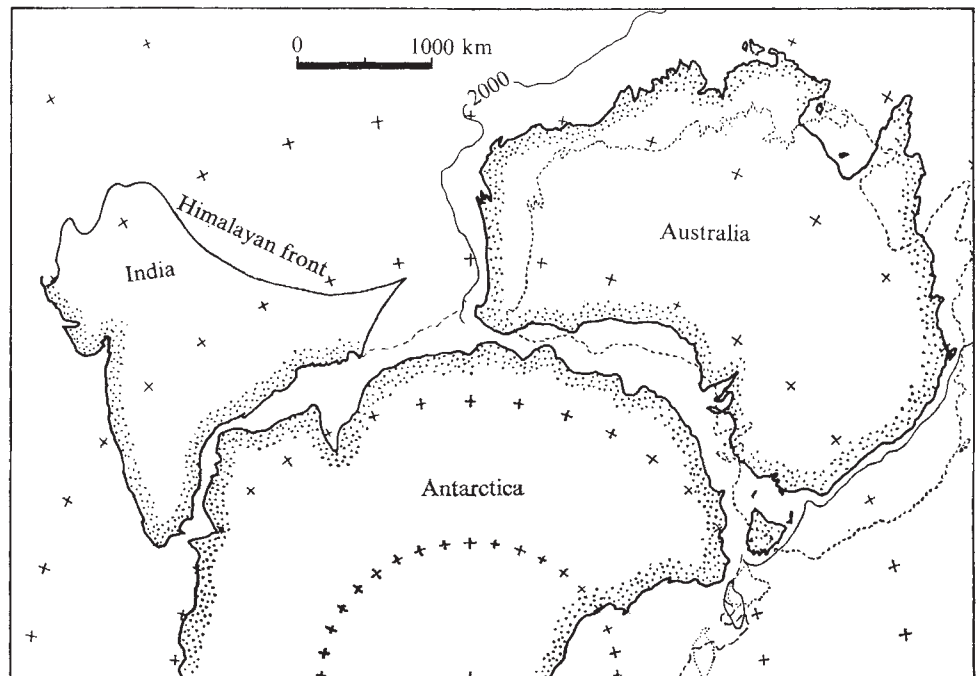


Kerguelen and Heard Islands on the northern section have been the site of recent volcanism¹⁶ although early Tertiary limestones occur on Heard Island¹⁷. Cenomanian chalk was cored from the flanks of the deeper southern section¹⁴. We suggest, therefore, that the northern section of Kerguelen Plateau has been formed since the Eocene, and that the southern part is a remnant of an older structure, possibly

a ridge that consisted of Broken Ridge, the southern section of Kerguelen Plateau and Naturaliste Plateau.

India moved rapidly northwards away from Antarctica from the late Cretaceous until approximately the time of anomaly 22 when the rate and direction changed markedly^{4,7,8}. At approximately this same time, Australia broke away from Antarctica¹⁸. The reasonably good match

Fig. 2 East Gondwanaland with India in the computer fit² with a computer fit⁴ of Australia as a dashed line.



of the fracture zones and magnetic anomalies on the India and Antarctica plates in Fig. 1 implies that at the time of anomaly 22, India and Australia became joined together as parts of the same plate and motion on the Ninetyeast transform fault ceased.

Sclater and Fisher⁷ in their model for the evolution of the eastern Indian Ocean used a computer fit⁴ between Australia and Antarctica for their reconstructions. This left a longer distance between the sequences of anomalies 22–32 on the India and Antarctica plates than in Fig. 1. As India and Australia are now on the same plate, Sclater and Fisher were forced to maintain a three-plate configuration between the times of anomalies 22 and 11 with right lateral strike-slip motion on the Ninetyeast transform fault. Thus the reconstruction in Fig. 1 suggests that this complexity is unnecessary.

Because the west coast of Australia seems not to have been open to the sea until the Jurassic¹⁹, several authors have suggested that the original Indian continental fragment extended to the west coast of Australia^{9–11}. The revised Australia–Antarctica fit brings Australia approximately 250 km closer to the reconstructed position of India and reduces the extent of 'Greater India' by this amount (Fig. 2). In this new reconstruction, the width of the portion of 'Greater India' between the locus of the present Himalayan Front and the margin of Western Australia is about 600–700 km. As the Himalaya are about 300 km wide and crustal shortening of about this much is suggested by geological and geophysical observations^{20–22}, we contend that essentially all of this Greater India could be absorbed in the Himalaya without the need for postulating the subduction of a large continental fragment beneath Tibet^{9–11}.

Thus the revised reconstruction of Griffiths¹ and Laird *et al.*⁶ not only improves the geologic fit between Australia and Antarctica but allows for a simpler model for the evolution of the east Indian Ocean⁷ and for a smaller amount of material to be absorbed in the Himalaya than had been supposed^{9–11}.

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Sensitivity of the homing pigeon to an Earth-strength magnetic field

THROUGH the use of a flight tunnel constructed within a controlled magnetic environment, it has been possible to train three pairs of homing pigeons (*Columba livia*) to discriminate between the presence and absence of a 0.5 gauss (Earth-strength) magnetic field. Successful discrimination was associated with flutter activity of the birds within the tunnel.

The ability of homing pigeons to return from an unfamiliar release site may depend upon a system of 'map and compass' navigation^{1–3}. Celestial sources of compass information have been demonstrated with pigeons^{4,5} and migratory birds^{6,7}. Sources of map information, however, have remained obscure^{2,3,8}. The occurrence of homing under a wide variety of experimental and natural conditions suggests that several environmental cues are involved.

Characteristics of the Earth's magnetic field, if detected, could participate in the formation of a complete navigational grid. Both the total field strength and the vertical field component (angle of inclination) are maximum at the poles and minimum at the magnetic equator. The angle of declination (between the geographic north and magnetic north meridians) shows complementary variation with longitude.

Early field tests of magnetic orientation were conducted by comparing the homing performance of birds with attached bar magnets to birds with attached non-magnetic equivalent weights⁹. Conflicting results were obtained under sunny test conditions, and no conclusions could be drawn.

Through experiments in which the birds' internal biological clocks were reset, it was found that the Sun provided only compass information^{4,5,10}. Thus, a new series of magnet experiments was prompted by the demonstration of Keeton¹¹ that pigeons could orient and home from an unfamiliar location under complete overcast by using Sun-independent cues. The vanishing bearings of experienced birds released with magnets under overcast were found to be randomised¹². In sunny conditions, however, only experienced birds released at greater distances or first-flight youngsters were disoriented. With a pair of battery-powered Helmholtz coils, Walcott⁴ was able to induce more uniform vertical magnetic fields through the pigeons' heads. By reversing the battery polarity, it was possible to change the inclination of the resultant field and obtain predictable and reversible orientation responses¹³. Normal fluctuations of less than 70 nT (1 gamma = 10⁻⁹ T) in the Earth's magnetic field have also been shown to correlate with the orientation performance under Sun of both pigeons¹⁴ and Singbilled gulls¹⁵. In pigeons, attached magnets have been used to suppress the correlation¹⁶. Walcott (private communication) has found the bearings under Sun of birds released near natural magnetic anomalies to be randomised. Together, these observations suggest that the Earth's magnetic field has a role in the normal navigation of homing pigeons.

With specially designed cages, it has been possible to record the orientation preferences of migratory birds in response to various environmental cues. W. and R. Wilt-schko^{17,18} and Emlen *et al.*¹⁹ have obtained predictable and biologically appropriate responses from several species of birds to natural and artificial magnetic stimuli. Attempts thus far to record the meaningful orientation of pigeons within cages have been unsuccessful, however, and other behavioural techniques have been applied. Using classical conditioning of the pigeon's heart rate, Kreithen and Keeton have demonstrated a sensitivity to polarised light²⁰ and to small changes in barometric pressure²¹. Reille²² reported some positive results in response to magnetic

stimuli; however, Kreithen and Keeton²³, Beaugrand²⁴, and Bookman (unpublished observations) have been unable to repeat or extend his findings. Attempts by several investigators to use operant conditioning with magnetic stimuli have also been unsuccessful²⁵ but largely unpublished. Previous laboratory testing has therefore failed to provide a conclusive demonstration of magnetic sensitivity in the homing pigeon, leaving the suggestive results of outdoor test releases unconfirmed.

Earlier laboratory experiments, particularly those using classical conditioning, required that the pigeons be restrained from active movement. The design and construction of a flight tunnel (Fig. 1) permitted greater activity, including voluntary flight, during the testing period.

Training was undertaken to determine if pigeons could learn to discriminate between a 0.5 G induced field and the 0.02 G background field. During a preliminary period of adaptation to the testing apparatus, it was found that mated pairs of birds were much more active within the tunnel than single birds. Therefore, intensive training was undertaken with three mated pairs borrowed from several lofts.

After 2 weeks, the birds' behaviour had been sufficiently shaped to allow the recording of data. Each trial began by uniformly releasing a pair of birds at the tunnel entrance. The pigeons then travelled the length of the tunnel and were trained to enter the correct feeding box associated with each magnetic field condition. The sequence of magnet coil activation was determined at random and set before the start of each trial. To serve as a positive reinforcement, food was available only from the correct feeding box. Incorrect responses were punished by confining the bird within the box for 30 s before allowing it to enter the correct box. All trials ended with the recovery of both birds from the correct box. Only the first response of the first bird was recorded, as the second bird did not respond independently. For the duration of testing, the birds were housed in cages within a naturally-lighted and ventilated room, and maintained within 10% of their *ad lib.* weight.

Two basic patterns of response were observed during adaptation and training of the pigeons. After landing, the birds would either walk the length of the tunnel or engage in a period of spontaneous 'flutter' activity before entering a feeding box. Flutter consisted of sustained hovering, jumping, rapid turning and short flights. Trials with at least 3 s of continuous flutter activity were recorded as 'with flutter', the remainder were designated 'no flutter'. A summary of the data is presented in Table 1. The cumulative performance record for one pair of birds is detailed in Fig. 2.

The data indicate that a statistically significant discrimination had occurred. This was even more apparent when the trials were separated according to flutter activity.

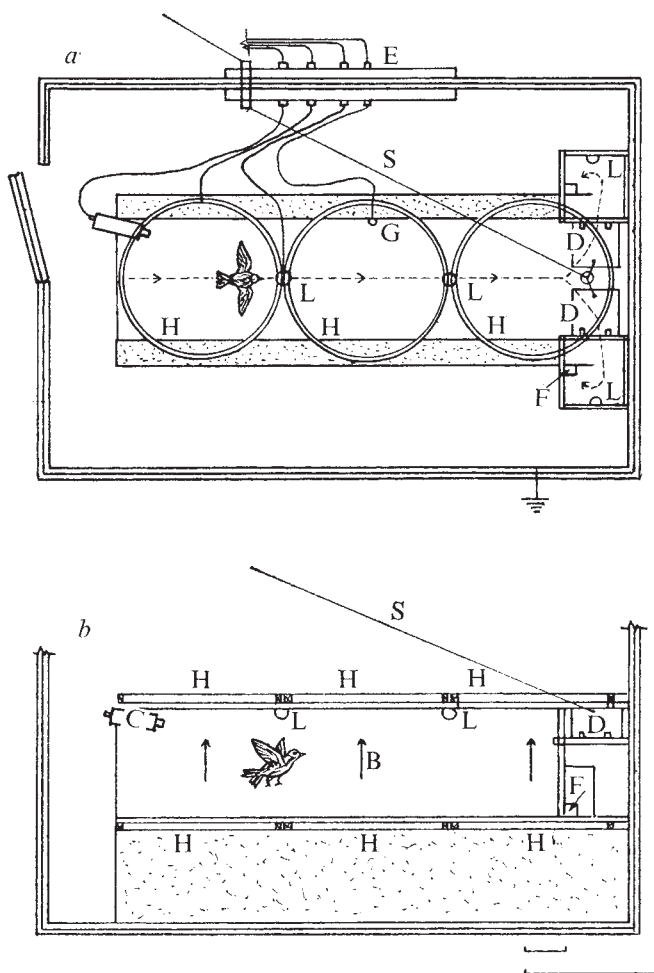


Fig. 1 Pigeon flight tunnel. Top view (a) and side view (b) of flight tunnel as constructed within mu-metal shielded room. Shielding reduced the natural magnetic field to a level of 0.02 ± 0.01 G. Three pairs of Helmholtz coils (H) were connected in series and arranged to induce a vertical magnetic field (B) along the length of the tunnel in as uniform a manner as possible. Each coil was wound with 150 turns of 12 gauge copper wire. Approximately 7.5 V d.c. at 100 mA was required to induce a 0.5 G (Earth-strength) magnetic field, as measured from a central probe (G). When the coils were not in use, the current was diverted across an external equivalent resistance. Two feeding boxes with concealed food bins (F) were located at the far end of the tunnel. For each trial, food in one bin was accessible while food in the opposite bin was covered by an aluminium screen that prevented access. Birds could be confined within the boxes by remote-controlled doors (D) which were released by string (S). Illumination was provided by 12 V d.c. lamps (L). A closed-circuit television camera (C) and electrical patchboard (E) allowed monitoring and control to proceed from a separate room which housed the power supplies, gaussmeter and television monitor. Scales: upper, 1 ft; lower, 1 m.

Table 1 Summary of flight tunnel training results

Pigeons		No. of trials	No. correct	Percentage correct	Probability*
Cornell pair	Total	150	101	67.3	< 0.0001
	With flutter	89	68	76.4	< 0.0001
	No flutter	61	33	54.1	NS
Richards pair	Total	129	89	69.0	< 0.0001
	With flutter	61	50	82.0	< 0.0001
	No flutter	68	39	57.4	NS
Walcott pair	Total	36	23	63.9	< 0.15
	With flutter	16	13	81.2	< 0.025
	No flutter	20	10	50.0	NS

*Two-tailed binomial probability for observed distribution.
NS, Non-significant (random) distribution.

In all cases, the 'with flutter' trials were statistically non-random while the 'no flutter' trials were random. Performance of the birds was not below the chance level in any category. The only factor altered from one trial to the next was the applied magnetic field. Although attempts were made to prevent any inadvertent cues (such as power supply hum), the influence of any such cues on discrimination would probably be independent of flutter activity. Therefore, the finding of significant differences between trials with and without flutter suggests that inadvertent cues or experimenter-induced bias did not influence the results.

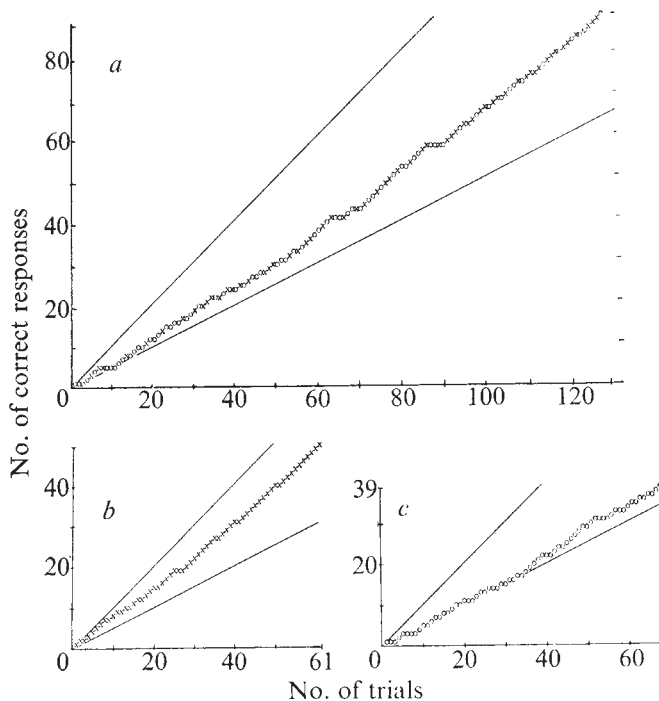


Fig. 2 Richards birds: cumulative record. Graphs for one pair of birds showing the number of trials with correct responses against the total number of trials. Only the response of the first bird to make a response was recorded for each trial. Lines indicating the 50% (chance) and 100% (perfect; upper line) levels of performance have been added. *a*, The total trial sequence is shown. Note that a significant discrimination had occurred and that 'no flutter' (○) and 'with flutter' (×) trials were distributed throughout the testing period ($P < 0.0001$). *b*, 'With flutter' (×) trials graphed separately ($P < 0.0001$). *c*, 'No flutter' (○) trials graphed separately (non-significant). Significant discrimination only occurred in the 'with flutter' trial sequence.

Thus, it seems that homing pigeons have a magnetic field sensitivity that can be demonstrated in the appropriate laboratory setting. Unlike other more conventional senses, the magnetic sense is somehow associated with flutter activity. Fluttering is also seen in successful experiments with caged migratory birds¹⁷⁻¹⁹. Although fluttering may be directly involved with the mechanisms of perception, it is just as likely to reflect a more general sensory activation that accompanies orientation. The biological mechanism of magnetic sensitivity in birds remains unknown, but may include paramagnetic, inductive or ferromagnetic interactions. The testing of pairs of birds in a flight tunnel is a straight-forward laboratory technique that could be applied to these and other unanswered questions in orientation research.

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Autogeny in saltmarsh mosquitoes induced by a substance from the male accessory gland

OVARIAN maturation by mosquitoes and other haematophagous Diptera without a prior blood meal is called autogeny, while the term anautogeny applies to those cases where blood is necessary. Many populations of the saltmarsh mosquito, *Aedes taeniorhynchus*, contain both autogenous and anautogenous females¹⁻². Moreover, within this species there are two types of autogenous females: (1) those that need a stimulus from mating to produce eggs autogenously and (2) those that lack this requirement³. We report that autogenous egg production associated with mating is triggered by a substance from the male accessory gland. The stimulation of normal oviposition behaviour and the termination of sexual receptivity in the female mosquito are also caused by a male accessory gland substance which is introduced into the female during mating^{4,5}. Both of these responses are uniformly exhibited by all females of several species⁶.

To demonstrate the influence of the accessory gland on ovarian maturation, we injected non-blood-fed, virgin females with tissue and fluid obtained from sonicated male accessory glands. This suspension was prepared by placing the glands of 250 virgin males in 1 ml of saline solution⁷. Since each female was injected with 1 μ l of suspension, our standard dose was equivalent to one-fourth of a pair of male glands. Males were at least 5 d old before paired accessory glands were removed. We used two closely related species, *A. taeniorhynchus* and *A. sollicitans*, both as the recipients and the sources of glands. All experiments with *A. taeniorhynchus* involved either the Rutgers stock or the Lake Charles strains. There are no laboratory colonies of *A. sollicitans*. Thus, we used F₁ offspring of wild adults collected in Indian River County, Florida. Procedures for rearing and maintaining mosquitoes followed those of O'Meara and Evans⁸.

Only a few non-injected and saline injected females developed eggs (Table 1), but over half of the *A. taeniorhynchus* injected with male accessory gland fluid (MAGF)

produced eggs in the absence of a blood meal. The ovarian stimulant associated with the gland seemed to be non-specific since *A. taeniorhynchus* responded in a similar fashion to material from either *A. sollicitans* or *A. taeniorhynchus*. The observed frequencies of autogeny in both the Rutgers stock and the Lake Charles females injected with accessory gland material were equivalent to the levels obtained in our previous studies where the females were allowed to mate³. During all treatments the vast majority of the *A. sollicitans* females remained anautogenous (Table 1). Of the 1,005 *A. sollicitans* females examined only four were autogenous. The rare occurrence of autogeny in *A. sollicitans* evidently is independent of MAGF and probably any other factor associated with mating.

The ovarian maturation associated with MAGF injections was not an artifact caused by just a general increase in the total protein concentration of the mosquito's haemolymph. Neither human serum nor protein from the fat bodies of male *A. taeniorhynchus* stimulated autogenous egg development (Table 1). For the various treatments, the amount of protein injected into each female was as follows: 10% human serum, 10.0 µg; 5% human serum, 5.0 µg; MAGF (*A. taeniorhynchus*), 0.25 µg; male fat bodies, 0.25 µg. The amount of protein that the females obtained from MAGF was quite small. Thus, most of the protein for autogenous egg production must have come from the female's internal reserves which were accumulated during the larval stage.

The ability of male accessory glands to induce ovarian development is acquired sometime after adult emergence. Females show no ovarian response when they are injected with MAGF from very young males (<1 h old).

At least three hormones are involved in the overall regulation of ovarian development in mosquitoes^{9,10}. Both autogenous and anautogenous females seem to use the same general endocrine pathway. Differences have been discovered in the mechanism controlling release of egg development neurosecretory hormone (EDNH) (ref. 11), however. Some *A. taeniorhynchus* females need no external stimulus

to trigger the release of EDNH and thus they begin autogenous ovarian maturation even before they are mated. Blood feeding will also trigger the release of EDNH and, possibly, MAGF does the same.

The association of MAGF with autogeny might represent another matrone-induced phenomenon. Matrone, a male accessory gland substance, is responsible for stimulating normal ovipositional behaviour and for terminating sexual receptivity in the female^{12,13}. These responses to matrone, however, are normally exhibited by all females in a mosquito population, whereas only a portion of the *A. taeniorhynchus* females in a population respond to the male accessory gland factor by producing eggs.

In *A. taeniorhynchus*, geographical variation in autogenous fecundity seems to be controlled by polygenic factors¹. Results of preliminary crossing and selection experiments indicate that other genetic factors control the occurrence of the various types of autogenous and anautogenous females. Attempts to identify the major genetic components involved in the regulation of autogeny are in progress.

The occurrence of two types of autogenous females within a mosquito population has been observed in other species besides *A. taeniorhynchus*. Working with the arctic species, *Aedes impiger* and *Aedes nigripes*, Corbet¹⁴ found both obligate and facultative autogeny. Females with the facultative type of development were potentially anautogenous for several days after emergence. But, if they did not succeed in obtaining a blood meal, then they switched to an autogenous mode of egg maturation. Corbet¹⁴ postulated that this change in the female's reproductive strategy was a response to the female's energy reserves, although no experimental study has produced evidence to support this theory.

Clearly, male-induced ovarian maturation represents a facultative form of autogeny and the switch from potentially anautogenous to autogenous development is mediated by a chemical from the male accessory gland. Most *A. taeniorhynchus* females are refractory to insemination for at least 36 h after emergence^{15,16}. The average duration of this refractory period is about 72 h in both the Lake Charles and Rutgers stock strains. Where insemination is a prerequisite for autogeny, the refractory period would contribute to the delay in the onset of ovarian maturation. As long as the ovaries remain undeveloped the females continue to be active blood-feeders and the fecundity in the initial egg batch is greatly enhanced in those mosquitoes that feed at this stage. Such a flexible system involving autogeny enables the females to capitalise on the availability of suitable hosts while at the same time it provides a means whereby egg maturation can occur in the absence of any host. Additional studies are needed to determine if mating and MAGF influence ovarian maturation in other mosquito species.

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Table 1 Effect of injected male accessory gland fluid (MAGF) from *A. taeniorhynchus* (AT) and *A. sollicitans* (AS) on females of each species

Treatment*	Females		Eggs per female†
	No. examined	% Autogenous	
<i>A. taeniorhynchus</i> (Lake Charles)			
MAGF-AT	556	53.1	22.1
MAGF-AS	218	62.8	21.5
Saline	484	4.5	20.8
Non-injected	615	2.6	25.4
<i>A. taeniorhynchus</i> (Rutgers stock)			
MAGF-AT	442	55.9	18.7
MAGF-AS	139	66.9	17.4
Saline	583	3.9	18.1
Human serum (10%)	73	1.4	15.0
Human serum (5%)	72	0.0	—
Fat bodies‡	72	1.4	11.0
Non-injected	950	1.9	19.2
<i>A. sollicitans</i>			
MAGF-AT	208	0.0	—
MAGF-AS	274	0.4	15
Saline	293	0.7	10
Non-injected	230	0.4	31

Sexes were separated within 3 h after adult emergence. All tests were conducted with virgins who had access to 10% sugar solutions. Females were denied oviposition sites and they were considered autogenous only when at least one ovarian follicle had completed maturation to stage V. Males were at least 5 d old before their accessory glands were removed. If mated the autogeny rates were about 50% in both the Rutgers stock and the Lake Charles strains (see ref. 3).

*Females injected with 1 µl of solution 2-3 days after emergence and then examined for ovarian development 7 days later.

†Mean no. of eggs per autogenous female.

‡Fat bodies from the abdomens of male *A. taeniorhynchus*.

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Influence of the seminal plug on mating behaviour in the garter snake

PHEROMONES are used by a wide variety of animals to identify the social status, sex and reproductive state of conspecifics¹. In snakes, olfactory cues are involved in prey selection and trail following^{2–4}, recent experiments indicate that female sexual attractivity in the garter snake *Thamnophis sirtalis* is oestrogen-dependent and that sexually active males discriminate between, and preferentially court, oestrogen-injected females^{5,6}. We report here that in the closely related species, *T. radix*, sexually active males do not court recently mated females. This discrimination is based on the presence of a seminal plug normally deposited by the mating male during copulation, and the mating plug exerts an inhibitory effect on the courtship activity of other males.

Courtship and copulation in *Thamnophis* are readily identifiable behaviours. Male approach is visually guided^{7,8} and courtship behaviour is mediated by tongue-flick delivery of odour molecules to the vomeronasal organs as in trail-following^{2,3,7,9}. The male orients to and approaches the female, tongue flicking rapidly^{5–7}. At first contact, the male frequently drapes his tongue over the female's back, the probable source of the sex attractant⁷. If the female is sexually attractive, the male will begin to 'chin rub', a behaviour in which the male places his chin on the female's back and repeatedly traverses her length. The male then aligns his body beside the female's with his head resting behind the female's. Palpations with the posterior third of the body follow as the male slides back to oppose the cloacae. Female sexual receptivity is characterised by lifting the tail and gaping the cloacae¹⁰, stimulating the male to evert and intromit a single hemipenis. Snake hemipenes are complex structures with numerous calyces and spines, believed to have a role in the copulatory lock¹¹. Copulation in the laboratory has an average duration of 91 min (range: 28.22–188.73 min) and is very vigorous. Sperm transfer is followed by the deposition of a gelatinous material produced by the renal sex segment, forming a copulatory plug^{8,12,13}; it has been suggested that the plug functions to reduce sperm leakage and prevent insemination by other males (ref. 13, but see ref. 14). Following separation, the male is sexually refractory for at least 24 h.

To assess the effect of mating on female sexual attractivity, attractive females were retested for sexual attractivity after having been allowed to mate. Female attractivity decreased markedly after mating and the decline was immediate and long-lasting (Table 1). Most non-mated males failed to court mated females even though they had courted the same female immediately prior to the female's copulation.

To determine the role of the mating plug in the post-mating decline in female attractivity, the mating plug was expressed and the female's cloaca laved with 10 ml distilled water immediately following mating. Females were then retested for attractivity (see Table 1 for protocol). Females with mating plugs removed remained attractive to sexually active males; seven of eight females were courted during the retest.

As in lizards and various other vertebrates and invertebrates (reviewed in ref. 15) mating in *Thamnophis* seems to inhibit further female sexual receptivity. In lizards, this decline in receptivity following mating is immediate and a consequence of male intromission¹⁵. In the present experiment, recently mated females which had had the mating plug removed were courted vigorously but failed to raise

the tail and gape the cloaca in response to male palpation for at least 48 h after mating; thus, they were attractive but not receptive.

To establish further that the post-mating decline in female attractivity was related to the mating plug, a third experiment was conducted in which cloacal material from both recently mated females and from courted but unmated females was collected and applied to sexually attractive females. Results demonstrated clearly that the mating plug is responsible for the loss of female attractivity following mating (Table 2). Females rubbed with cloacal material from unmated females or distilled water were actively courted; however, most females with cloacal material from recently mated females were not courted. Most females were courted after the applied material was removed with a wash of alcohol followed by water.

As these experiments were in progress, it became apparent that the behaviour of sexually active males changed dramatically following exposure to a mated female. To assess the effect of exposure to a mated female on subsequent male courtship, recently mated females were introduced into cages containing proven sexually active males (as determined by consecutive male courtship of two injected but unmated females immediately before presentation of the mated female). After a 5-min test with a mated female, the unmated females were reintroduced in order and male courtship of each scored. Exposure to a mated female precipitated a marked reduction in male courtship activity; if exposed to a mated female, previously sexually active males (nine of eleven) did not court females they had just courted. The duration of this effect varied between males.

T. radix exhibit a reproductive cycle typical of high latitude species. In the spring, breeding is restricted and compressed into the first days after emergence from the winter hibernaculum^{12,16}. Males emerge several days before females¹⁷ but do not leave the area. On emergence, females are courted *en masse* by the males in the immediate vicinity; mating 'balls' of 100 or more males courting a single female have been reported. It is not known if this mass courtship facilitates female receptivity. It appears that once a single male intromits, the unsuccessful males abruptly leave the copulating pair^{7,12,18}, further, recently mated females are usually not courted¹².

Table 1 Influence of mating on female attractivity in the garter snake *Thamnophis radix**

Treatment	N	No. of females courted
Pre-mating	17	17
Post-mating:		
5–20 min	9	0
24 h	16	2†
36–48 h	11	3†

*Not all females were retested for attractivity at all intervals post-mating. All behavioural tests were 5 min in duration. Males were considered to be sexually active if they courted the female uninterrupted for 30 s; a female courted consecutively by two males was considered sexually attractive. Precautions were taken to avoid transferring odours through handling. Animals were purchased in September from a commercial supplier in Wisconsin. Snakes were handled 1–2 ml of a meat-egg-vitamin mixture each week. Water for drinking and soaking was available *ad libitum*. Male snakes were kept individually in wire mesh enclosures (61 × 22 × 77 cm). Unmated females were housed in groups of six in wire cages; mated females were separated and caged individually. Cages containing males and testing cages were inside Sherer Gillett RI-48-LLTP environmental chambers programmed to provide a 14:10 L:D photic cycle with a corresponding thermal regimen of 32:23 °C; females were exposed to a 14:10, 27 °C daily photothermal cycle. All snakes were intact and received the following hormone treatment. Males were induced to a sexually active state by subcutaneous (s.c.) implantation of a 2.0 cm silastic capsule (0.15 mm inside diameter) containing approximately 16 mg of free testosterone. Females received daily s.c. injections of oestradiol benzoate^{5,6} (50 µg per day); injections were discontinued on the eighth day. N, no. of animals tested.

†In two cases, post-mating courtship involved the same test male.

Table 2 Role of mating plug in mating-induced decline in female attractivity

Treatment	N	No. of females courted
Pre-test:		
Untreated	18	18
Test:		
Cloacal wash		
mated female	7	1*
unmated female	7	7
Distilled water	4	4
Post-test:		
Alcohol-water wash	18	15†

Females were tested for sexual attractivity using protocol described in Table 1. In the first of three consecutive courtship tests, the sexual attractivity of the females was established. Cloacal material from recently mated females or unmated females, or distilled water, was then applied to the dorsal surface and the females were retested for attractivity. Females were then washed with alcohol (90%) followed by distilled water to remove the applied substance before being retested for attractivity. N, no. of animals tested.

*Male courtship weak.

†Of the three non-courted females, two were not courted during the post-test period because of the decline in male courtship behaviour as a result of the exposure to mating plug material (see text).

These field observations of male dispersal from the mating pair suggest that the mating male deposits an odour(s) during copulation which communicates to other males the mated status, and hence non-receptivity, of the female. Thus, it is possible that the mating plug may, in addition to aiding in sperm retention, enable other sexually active males to determine whether a particular female has mated recently.

The experimental finding that exposure to odour(s) secreted during copulation (presumably by the male's urogenital system) results in sexually active males 'losing interest' in unmated, sexually attractive females, however, suggests an alternate explanation for male dispersal from the mating ball. In those insects having dense mating aggregations in which the probability of multiple inseminations is high (intense sperm competition), mechanisms have evolved which reduce the likelihood of multiple inseminations¹⁹. It is possible that, in *Thamnophis*, the odour(s) deposited by the mating male maximise the male's reproductive success by subsequently decreasing female attractivity as well as temporarily removing other males from the breeding population (that is, while the recently mated male is sexually refractory and unable to mate). Male dispersal from the mating ball, therefore, could be a consequence of unsuccessful males avoiding these odour(s). The demonstration and clarification of either or both of these postcopulatory adaptations must await detailed field studies of *Thamnophis* sexual behaviour.

Field observations also indicate that females leave the vicinity of the hibernaculum soon after emergence and copulation¹⁷. Since copulation is prolonged and conspicuous in *Thamnophis*, the mating pair is presumably very vulnerable to predation at this time. Mating-inhibition of further female sexual attractivity and receptivity, therefore, may act along with the female's departure from the hibernaculum area to minimise the female's vulnerability to predation (see also refs 15, 17).

Although our findings may be due to the restricted testing conditions, they demonstrate that sexually active males do not court sexually attractive females after prolonged or forced exposure to copulation odour(s). The specificity of this decline in male courtship behaviour as a consequence of exposure to a mated female is indicated by the observation that such males continue to investigate introduced snakes and trail-follow. It would thus be of interest to know whether this cessation of courting behaviour is due to a change in sensitivity at the peripheral or central level. In any case, the demonstration in verte-

brates that the mating plug of one male can influence the behaviour of other males toward other females is, to the best of our knowledge, unique.

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Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes

REPRODUCTIVE competition for females among males of a given species can take many forms, some of which (like mating plugs) may place constraints on the mating opportunities of males and intensify competition for available females. I have studied the reproductive behaviour of two garter snake species (*Thamnophis sirtalis* and *T. butleri*) in the field for 5 years. They do not exhibit pair-bonding, paternal care of offspring, territoriality, or intrasexual combat. Sexual selection in this mating system should favour males which compete to locate, court and mate with the maximum number of females¹. Yet I have observed numerous solitary female *Thamnophis* within the same time periods and areas in which other females were being vigorously courted, often by several males. I have previously observed that the anterior cloaca of any recently mated female *Thamnophis* contains a copulatory plug which occludes the oviductal orifices². The plug evidently is formed by the copulating male after sperm transfer. I interpreted this as a form of intrasexual competition in which the successfully copulating male makes the female temporarily unavailable to other males and reduces the likelihood of multiple inseminations. Some reports^{3,4} indicate that multiple inseminations are possible. I present here evidence from the field that male garter snakes recognise females with a copulatory plug and behave as if these females were unavailable.

The pattern of emergence from hibernation and mating has been described for *T. sirtalis* in Canada⁵⁻⁸, but my own observations differ in several important respects. Mating activity in southeastern Michigan is most intense on warm days in March and April immediately following the emergence of these snakes from hibernation, but before they disperse from the denning area. A denning area in Michigan is a collection of separate refuges, such as crayfish or rodent burrows, no one of which is used by all of the snakes at

that area. Males are conspicuous throughout the mating season, either courting or actively moving through the denning area evidently searching for females which might be emerging from any of the refuges. The emergence of females from hibernation is sporadic with individuals appearing on different dates. Females showing evidence (the copulatory plug) of recent mating do not immediately disperse from the denning area. These females, however, were never being courted when I encountered them. By contrast, those females which did not have a plug were usually being courted (Table 1).

The hypothesis that females with cloacal plugs are unattractive to males was tested in the field. Individual male snakes were given the opportunity to court first a female with a plug and then a female without a plug. The results (Table 2) clearly show that sexually active males can recognise and are disinclined to court females with plugs. All of these males subsequently courted the females without plugs.

Table 1 Observed mating status and courting activity of female garter snakes

	No. being courted	No. not being courted	χ^2
<i>T. sirtalis</i> without plug	21	6	27.2
with plug	0	19	
<i>T. butleri</i> without plug	14	3	9.9
with plug	0	4	

Values are the total number of females observed at the mating areas during the mating seasons from 1974 to 1976. Mating season is defined for each year as the time from the first observed courtship to the last.

A male can increase his opportunities for mating by minimising the time spent in unproductive courtship. It would be advantageous for a male to recognise and disregard females with plugs if plugs effectively prevent further inseminations. Among males in competition to locate unmated females, those that can recognise the altered mating status of a female as soon as possible should be favoured. Frequently, several males court a female simultaneously. When one mates, the unsuccessful males tend to disperse, presumably seeking other females, before their successful rival even finishes mating. I have seen this occur three times in the field and similar reports from the field⁹ and the laboratory^{10,11} exist. Even the successful male should soon be seeking other females. In at least four cases, males that I had observed mating were courting another female within

Table 2 Responses of male garter snakes to females with or without copulatory plugs

Species	N	Courted both	Courted neither	Courted only female with plug	Courted only female without plug	Probability
<i>T. sirtalis</i>	11	2	0	0	9	<0.01
<i>T. butleri</i>	13	0	0	0	13	<0.001

The mid-body portion of a female with a plug was carefully set in front of each male. Both the head and anal portions of the female were held by hand to prevent her retreat into the undergrowth. If the male contacted the female with his tongue and initiated normal courtship by aligning his body along the coils of the female's body with his chin pressed tightly against her dorsal skin, his response was scored as positive. A response was scored as negative only after a male contacted the female with his tongue several times and then ignored her. As soon as a score was obtained for a female with plug, she was removed and the procedure was repeated on the same male using a female without a plug. In this matched-pairs design, each male served as his own control. Probabilities are for a one-tailed sign test.

an hour. More extensive data on males mating more than once in one afternoon have been reported¹².

The mechanism by which male garter snakes recognise the altered mating status of females with plugs remains unknown. In my experimental field trials, the males did not seem to behave differently toward either female until tongue contact was made. The males made no attempt to investigate the female's cloacal area (which was within my hand) before courting or rejecting her. This suggests that a chemical cue which is not physically associated with the plug itself may be involved. I doubt that the cue could be from the plug itself inasmuch as that is a male product. If a male were capable of producing any substance which directly caused other males to disregard a female, it would be most advantageous to use it at the beginning of courtship to avoid competition from courting rivals, rather than reserving it for use (in plugs) after mating was completed. This is not observed. If this potential advantage ever existed for some males, competing males that disregarded male odours and courted anyway would be favoured. Alternatively, plugged females could find it advantageous to 'announce' their altered status, since courting aggregations may be just as conspicuous to potential predators as they are to human observers. If fruitful mating is unlikely, females should not risk predation. Therefore, the female is probably responsible for producing the cue.

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Chameleons use accommodation cues to judge distance

ANY animal that adjusts the focus of its eye lens has monocular distance information potentially available from its focusing mechanism. Such accommodation cues are independent of size and context, and thus could provide an objective measure of distance. Ingle has proposed that such information is used by monocular frogs¹ and toads², both of which he has shown to be capable of estimating distance with one eye³. There is no direct evidence, however, that any animal perceives distance as a function of the plane of focus of the image. The experiments described here demonstrate that chameleons do so in estimating the distance of their prey.

It has not been shown that chameleons judge distance when feeding; however, their method of catching insects seems to be sufficiently exacting to require accurate distance assessment: the tongue is shot out with remarkable speed ($\sim 5 \text{ ms}^{-1}$) to hit and retrieve insects at distances up to 1.5 times body length⁴ (Fig. 1). The eyes of a chameleon normally move independently, each eye scanning (saccadically) over more than a hemisphere. While taking aim at an insect, however, both eyes are swivelled directly towards the target (Fig. 2), often prompting the assumption that these animals acquire distance information by some process of triangulation.

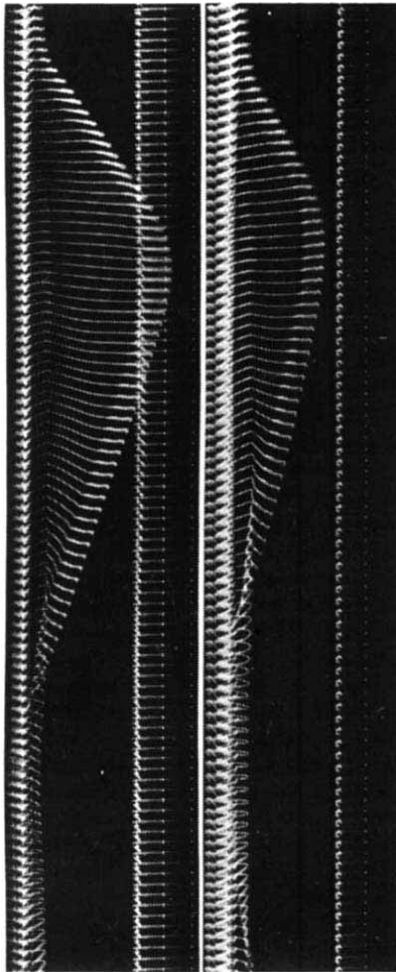


Fig. 1 Multiple exposure photographs of a chameleon attempting to capture an insect. 35-mm black and white film (Kodak Tri-X) was loaded into an oscilloscope camera, and the shutter left open. Illumination was provided by a strobe light flashing at 2.5 ms intervals. The camera motor drive was triggered by hand as the chameleon took aim and the film then moved past the shutter at 16 inches s^{-1} , thus separating the frames. The chameleon is on the left and is viewed from the side; only his head is visible. The insect he is attempting to catch is on the right, mounted on the end of a wooden stick (hence the series of horizontal bars on the right). The scale is given by the marks on the stick (5-cm spacing). Time moves down the page. At the top the chameleon's tongue can be seen protruding from its mouth as it takes aim. Then the tongue can be seen shooting across the gap towards the insect. *a*, The chameleon is wearing lateral prism spectacles (see Figs 2 and 3) and the tongue passes freely by the insect, before being retracted. *b*, In contrast, when the animal is wearing negative lens spectacles (see Fig. 3), the tongue never reaches the insect. This undershoot is predicted from the hypothesis; but naturally occurring undershoots in healthy adult chameleons are extremely rare (less than 1 per 250 attempts).

Chameleons were filmed while attempting to catch insects at various distances. The species used was *Chameleo jacksoni*, which has horns from which opaque screens, lenses and prisms could be suspended such that the animal was required to look through them as it took aim (see Fig. 2). This made it possible to manipulate what the chameleon was seeing while it was taking aim.

If chameleons judge prey distance, then one might expect some quantitative aspect of their behaviour to correlate with the range of the insect (the object distance, D_{obj}). Occasionally, a chameleon will miss the target, and it is then possible to measure the maximum distance the tongue reaches (maximum tongue extension, S_{max}). This unobstructed tongue flight can be produced consistently by providing the animal with an illusory target (Fig. 3*a*). The results of a series of such binocular shots are shown

in Fig. 3*b*. There is a positive correlation between the behavioural measure (S_{max}) and the distance of the insect (D_{obj}). A similar series was filmed of animals feeding monocularly, and a similar correlation was found to exist (Fig. 3*c*). Thus chameleons do judge prey distance both monocularly and binocularly, as in both cases they modify their behaviour according to the target distance.

Chameleons have a focusing mechanism⁵, so in theory they could use accommodation cues to estimate distance monocularly (or binocularly). When the ciliary muscles of the eye are at rest, parallel rays of light are brought into focus on the retina⁶. As an object is brought towards the eye, however, at some critical distance the image will no longer be in focus, and the lens must subsequently be deformed continuously to maintain a clear retinal image as the object moves closer. Within this accommodating range, the degree of lens deformation, or the motor command used to effect that deformation, could give a measure of the distance of the object fixated.

It is possible to test whether chameleons use accommodation cues while feeding by altering the plane of focus of the prey. For instance, the image of an insect viewed through a biconcave (negative) lens lies closer to the animal than the insect itself. Thus if an animal estimates distance as some function of the plane of focus, it would undershoot in this situation. Conversely, it would overshoot if wearing biconvex (positive) lenses. For the animals for whom control data exist, it is possible to calculate quantitatively their predicted behaviour wearing spectacles with lenses of any strength. The curves drawn above and below the regression lines in Figs 3*b* and 3*c* show the expected deviations from normal behaviour for the particular focal length lenses used in the experiments. There is a close fit between the data obtained experimentally and the behaviour predicted under the hypothesis that chameleons estimate prey distance as a function of the plane of focus.

None of the other recognised cues to distance gives



Fig. 2 A chameleon wearing spectacles. Chameleon eyes normally move independently, each eye scanning (saccadically) over more than a hemisphere. When taking aim at an insect, however, both eyes are swivelled directly towards the target, and chameleons always aim and shoot their tongues straight ahead. Thus spectacles can be positioned so that the animal must look through them while taking aim.

predictions at all comparable with those from the accommodation theory. Indeed, of the monocular cues, motion parallax, retinal image size and context cues such as texture gradients and perspective, all change in such a way that the predicted behaviour for an animal wearing lenses would be qualitatively the opposite of that actually observed. Neither of the binocular cues, retinal image disparity or the convergence angle of the eyes, is in theory altered by the spectacle lenses, although it could be argued that in practice the animal will probably not be looking through the optical centres of the lenses, so there will be prismatic effects that may alter the convergence of the eyes. Chameleons, however, seem unaffected by alteration of the binocular convergence angle (Fig. 3b).

In conclusion, binocular and monocular chameleons

perceive prey distance as some function of the plane of focus, and accommodation is not just used as a back-up cue when binocular information is unavailable.

If an animal uses its focusing mechanism as its primary source of distance information, one might expect the structure of the eye to have design features maximising the accuracy of such a cue. To maximise the detection of error in focusing, the eye should have good angular resolution; the organisation of the chameleon eye suggests that these animals, particularly for their size, have high acuity vision. Chameleons⁵ have a well developed fovea, comparable in general appearance (deep convexitate) with that of birds of prey^{5,7} renowned for their visual capabilities. Rochon-Duvigneaud's⁸ estimate of the chameleon foveal receptor cell density is probably much too high

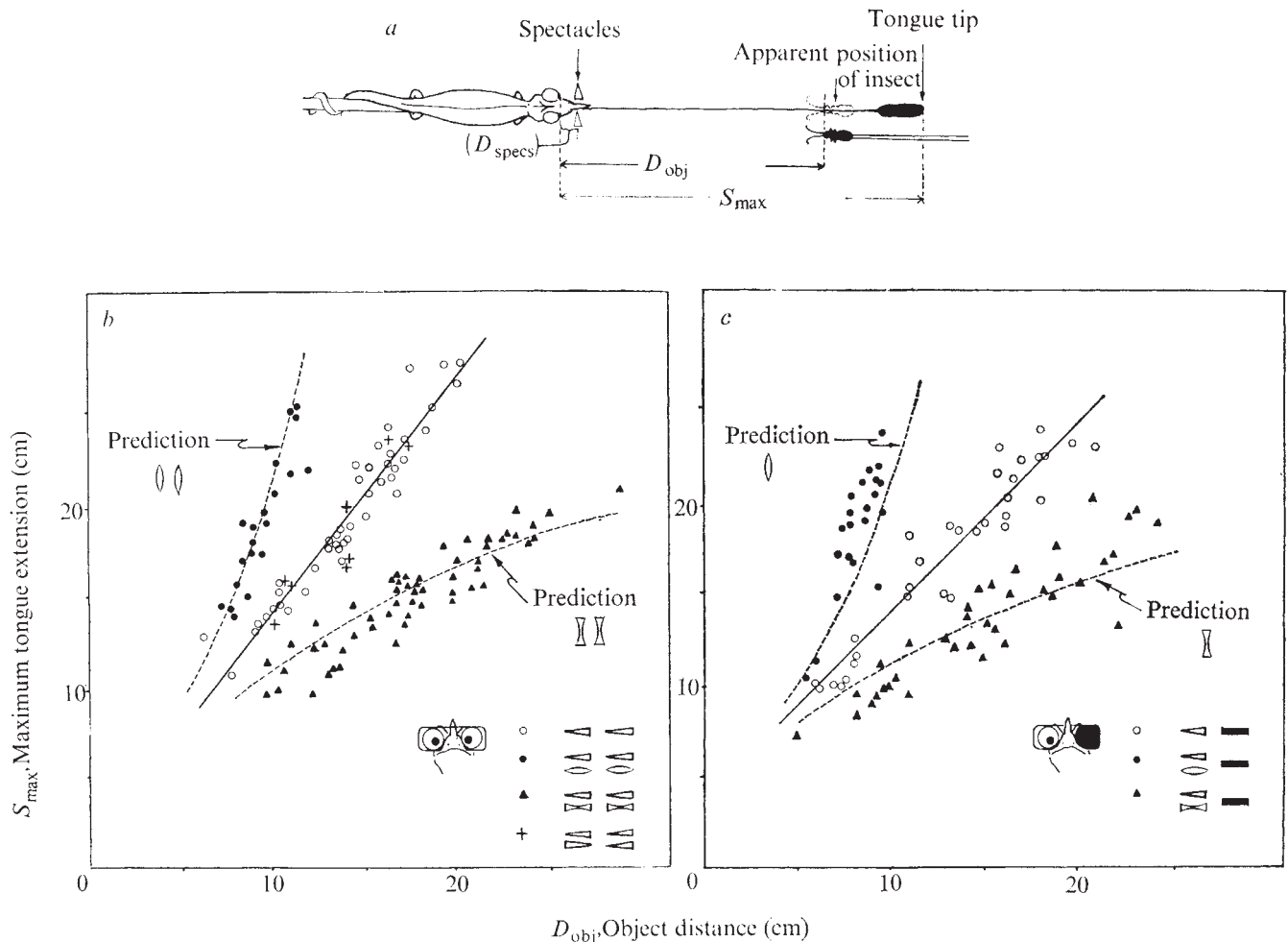


Fig. 3 a, A chameleon viewed from above while shooting at an illusory target. The image of the insect is displaced laterally by thin prism spectacles, and the tongue in consequence passes by to the side of the real insect. The object distance (D_{obj}) is defined as the distance between the near side of the insect and the chameleon's pupils, measured on the film frame just before the tongue is fired. The maximum tongue extension (S_{max}) is defined as the distance between the pupils and the tongue tip, measured on the film frame where the tongue is maximally extended. **b, c**, The relationship between the range of the insect target and the maximum tongue extension for binocular (**b**) and monocular (**c**) animals wearing spectacles (shown in section on the figures). Each point represents one attempt by one of the three animals tested. ○, Attempts where animals wore just lateral prism spectacles; —, regression lines fitted to the data giving equations of the form

$$S_{max} = a(D_{obj}) + b \quad (1)$$

— — —, Overshoots and undershoots predicted by the hypothesis for animals wearing lens spectacles. The distance of the focal plane from the chameleon's pupil ($D_{focal\ plane}$) can be calculated from the lens equation, the distance between the eyes and the spectacles (see Fig. 3a) being taken into account:

$$D_{focal\ plane} = D_{specs} + 1/[1/F - 1/(D_{obj} - D_{specs})]$$

where F is the focal length of the lens. Predicted S_{max} can then be calculated for different insect ranges, by substituting $D_{focal\ plane}$ for D_{obj} in equation (1). Using the appropriate values for a and b , curves were calculated for negative lenses (focal length -25 cm) and positive lenses (focal length $+22.3$ cm). These are the curves shown. ●, ▲, Data points obtained from animals wearing these lenses as spectacles. The close fit between the data and the predictions suggests that both monocular and binocular animals estimate prey distance as a function of the focal plane. In contrast, note the crosses (+) on Fig. 3b. These points were obtained from binocular animals wearing convergent prisms of sufficient angular deviation to predict dramatic undershoots if the animals were estimating distance as a function of the convergence angle of the eyes. Chameleons do not seem to triangulate to estimate distance.

(760,000 mm⁻²), but his ordering of chameleon cone densities (the greatest of all lizards) as lying between that of man and birds of prey, may be correct. Notice also how large the chameleon's eyes are compared with the head (Fig. 2). Absolute increase in eye size improves resolution if the receptor density remains constant, because with increasing eye size, a given object will project a larger image on the retina.

To maximise the error in focus itself, the system should have poor depth of field, which can be achieved with a wide aperture. Chameleons are capable of some pupillary constriction, yet in spite of high ambient light levels and the proximity of the insect (both of which would induce pupillary constriction in other animals⁸), chameleons maintain pupil dilatation while aiming (*f*/number of ~ 5). It is possible to hypothesise why chameleons should have evolved high acuity vision, for reasons other than to maximise the usefulness of accommodation cues to distance; however, the combination of good image resolution and poor depth of field is less easy to explain in other terms.

Walls⁶ expressed surprise that although lens-accommodating mechanisms are widespread within the animal kingdom, focusing mechanisms seemed to have failed to evolve into depth-sensing organs. I suggest that chameleons have evolved just such a system, and that it is possible that we may have underestimated the usefulness of accommodation cues to other animals.

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Stereopsis in toads

HARKNESS'S demonstration¹ that chameleons rely primarily upon accommodative cues to judge distance encouraged me to examine the mechanisms of depth vision in an amphibian (*Bufo marinus*). Unlike chameleons which have highly mobile eyes, toads make neither convergent nor fixating eye movements, so that, except for stabilising reflexes, their eyes are almost locked in their sockets². For simple forms of stereoscopic range finding it is an advantage to have immobile eyes because disparity measurements can be converted directly into estimates of absolute depth (Fig. 1a). One might therefore expect toads to use retinal disparity in judging the distance of their prey. Toads have the essential apparatus for stereopsis: there is a substantial binocular overlap both in front of and above the animal³, the inter-

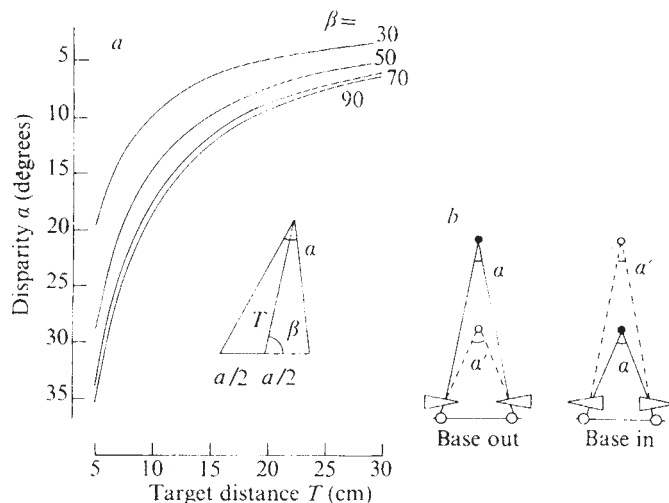


Fig. 1 *a*, Plot of the relation between disparity (α) and target distance (T) for a toad with an interpupillary distance of 3.2 cm. This value was typical of the toads tested. The relation between α and T depends on the lateral position of the target with respect to the toad's mid-line (β). However, this effect was negligible for the values of β that occurred in these experiments. *b*, Diagram to show how base-out and base-in prisms cause toads to misjudge the distance of a target. The dashed lines and open circles indicate the apparent lines of sight and positions of the target.

ocular distance is relatively large, and neural pathways bring information from corresponding areas on the two retinæ to the same region in both mid-brain⁴ and thalamic⁵ visual areas. My results suggest that toads do indeed make disparity measurements and should be added to the growing list of vertebrates known to achieve stereopsis (monkey⁶, cat⁷, owl⁸). In addition, toads have a mechanism for measuring depth within their extensive monocular field. Ingle has shown⁹ that frogs and toads, like chameleons, can estimate the distance of prey almost as well with one eye as with two, and the present data suggest that they do this by monitoring the accommodative state of their eyes when the prey is in optimum focus, as Ingle originally proposed⁹.

The accuracy with which toads assess the distance of their prey was determined from films of toads catching meal worms on the ground. Films were taken from above at 24 or 40 frames s⁻¹. When a toad snaps at prey within its frontal field it leans forward, extending its tongue. Two measures were derived from the films. 'Target-distance' (T) is the distance from a point midway between the eyes before the toad moves to the middle of the prey (or, when large dummy stimuli were used, a point 0.5 cm from its nearest edge). 'Snapping-distance' (S) is the distance from the same point between the eyes to the tip of the tongue at its fullest extent. Figure 2a shows that the tip of the tongue tends to overshoot the centre of the prey by about 1 cm. Thus a toad's snap includes an accurate estimate of target-distance.

The cues toads use to judge distance were studied by filming prey-capture at 40 frames s⁻¹ while toads wore lenses or prisms which distorted vision in their frontal field. Spectacle lenses alter the effective optical power of the eye so changing the normal relationship between the distance of an object and the lens position in which the image is in focus on the retina. Accordingly, a spectacle lens will systematically distort depth estimates that depend on the position of the lens. Negative spectacle lenses decrease the apparent distance of an object from u to $1/(1/u - 1/f)$, where f is the effective focal length of the spectacle lens at the principal point of the eye, and u the actual object distance. Prisms were used to assess the importance of stereopsis. Because amphibia have no fovea from which corresponding points in the two retinæ can be labelled, disparity is defined in a slightly unusual way, as the angle formed between lines connecting a target to the nodal point of

each eye (Fig. 1a). This is geometrically equivalent to the conventional meaning of disparity for conditions in which the lines of sight through the foveas are parallel. For a target that is straight ahead the relation between disparity and distance is given by the expression $\tan \alpha/2 = a/2T$, where T is the target distance and a the interpupillary distance. Prisms appropriately aligned change the apparent disparity of the target and thus alter the apparent target distance for an animal using stereopsis (Fig. 1b). Prisms that are base-out over each eye increase the apparent disparity (α') of the target by an amount equal to the summed angular deviation produced by the two prisms

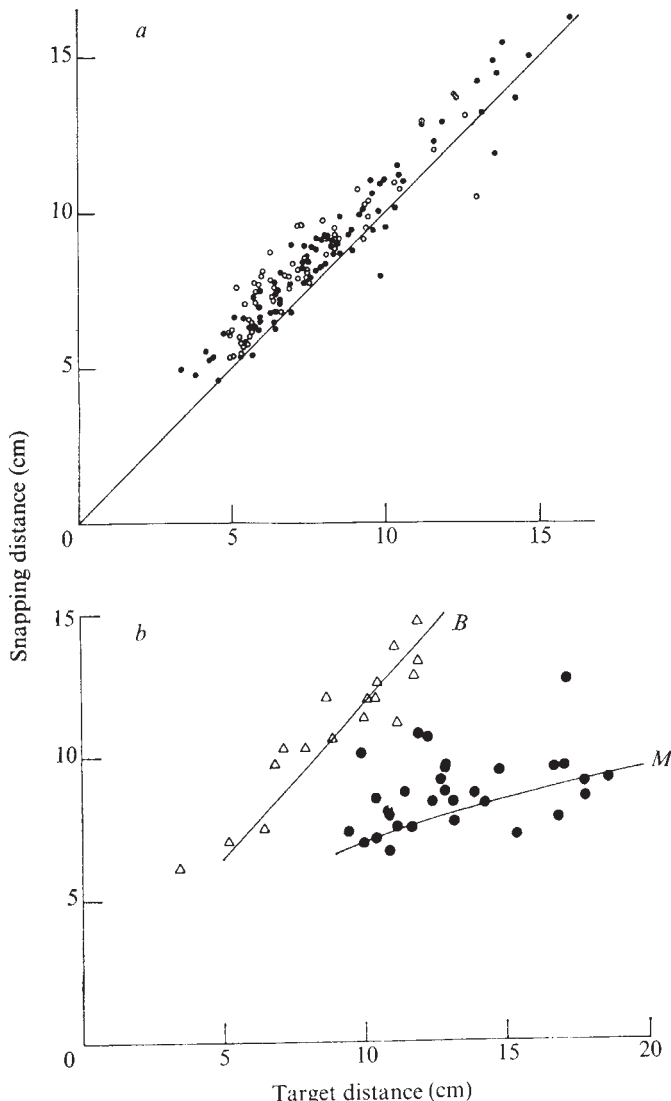


Fig. 2 *a*, Accuracy of prey capture in binocular and monocular toads snapping at meal worms moving on a dark substrate. Snapping distance is plotted against target distance. In this and subsequent graphs each data point represents a single snap. The toads tend to shoot about 1 cm beyond the centre of the target. ●, Data from seven binocular toads. ○, Monocular: data from a single toad which arrived from the animal dealer with one eye missing. *b*, The effect of -136 mm negative lenses on snapping distance. ●, Monocular: lens placed in front of one eye while the other eye is occluded. The line labelled *M* is the predicted snapping distance on the assumption that toads employ accommodative cues. △, Binocular lenses over both eyes. Line *B* is the predicted snapping distance (taking into account the prismatic effect of the lens), assuming that toads depend upon stereopsis and ignore monocular cues. When vision is monocular the toad appears to use accommodative cues, but when vision is binocular disparity measurements are more important. Data from one toad snapping at a yellow cylinder moved by hand in its frontal visual field.

at the eyes (2δ); that is $\alpha' = \alpha + 2\delta$. Base-in prisms have the opposite effect ($\alpha' = \alpha - 2\delta$). Thus, given the normal relation between S and T (Fig. 2a), the snapping distance can be predicted for a variety of lens and prism combinations. The fact that this technique can be applied successfully implies that snapping is an open-loop response in the sense that the toad decides how far it is going to extend its tongue before beginning the movement.

The importance of accommodation as a cue to distance is demonstrated by the effect of a negative lens ($f=136$ mm) on the snapping behaviour of a toad with one eye occluded (Fig. 2b). The toad then snaps short at prey in its frontal field. The data points fall around the line labelled *M* which predicts S on the assumption that distance measurements rely solely upon accommodative cues. If motion parallax or image size were significant cues to depth for monocular toads, negative lenses would cause overshooting. Some additional evidence also indicates that these cues are not important: (1) snapping accuracy is unaffected by prey size, and (2) when a target suddenly appears in front of a monocular toad, the toad usually snaps directly at it, without making any previous head movements. (One unexplained finding is that monocular positive lenses do not cause overshooting.)

Toads have a visual field of almost 360° within which they can detect prey. If the prey is close enough, toads snap at it directly without any prior orienting movement. Thus the toad's use of accommodative cues is not limited to a restricted region of retina. It must be less sensitive than that of the chameleon which always directs its fovea at prey before shooting out its tongue, so maximising its ability to detect and minimise blur¹.

The performance of toads wearing negative lenses over both eyes implies that disparity is the major cue to depth when targets are viewed binocularly. The snapping data then fall around the line labelled *B* (Fig. 2b) which is the predicted snapping distance on the assumption that disparity is the only measure of target distance. Tests with prisms reinforce this conclusion. Base-out prisms over both eyes cause the toad to undershoot by an amount which is greater the larger the angular deviation of the prisms (Fig. 3a), while base-in prisms cause overshooting. Prisms which are base-in over one eye and base-out over the other do not change the disparity of the target and accordingly have no effect on snapping distance. Instead the toad misses its prey laterally. Similarly, a base-out prism over one eye and an occluder over the other leads to lateral errors but no undershooting.

Base-out prisms produce a conflict between accommodative and stereoscopic information. Snapping distance is then a compromise between these two cues, heavily weighted in favour of stereopsis. Figure 3a shows that toads shoot beyond the point predicted by the apparent disparity of the prey by an amount that increases with prism strength (that is, with the magnitude of the discrepancy between the two cues). The relative importance of monocular and binocular cues was computed from snapping data taken from binocular toads wearing base-out prisms or combinations of base-out prisms and -136 mm negative lenses (Fig. 3b). For a wide range of prism strengths the data are well fitted by the linear expression

$$S = 0.94B + 0.06M + C$$

where C is a constant of about 1 cm. The expression holds for values of M between at least 8 and 30 cm and for values of B ranging from 5 to 15 cm. Thus, when vision is binocular, disparity is the predominant depth cue and accommodative cues are very largely ignored. Nevertheless, when vision is restricted to one eye, accommodation gives adequate distance information for targets in exactly the same part of the visual field (Fig. 2a).

If stereopsis is to operate over these distances without

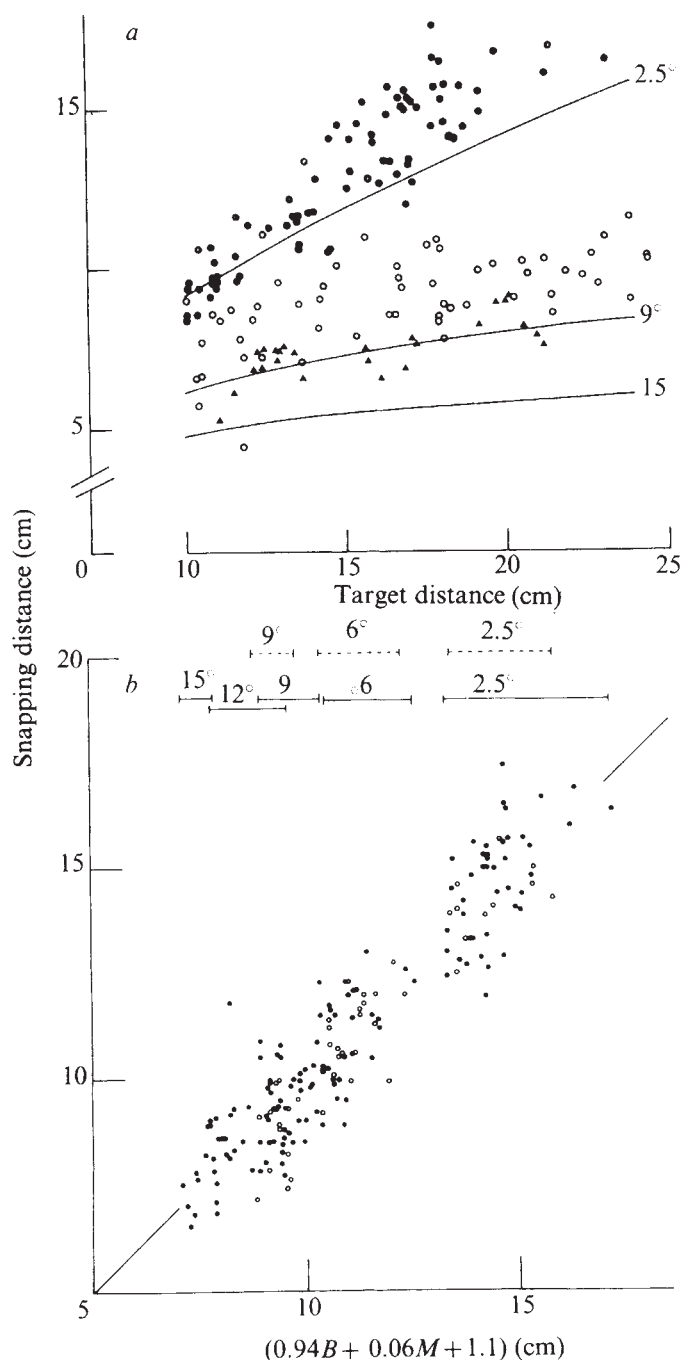


Fig. 3 *a*, Plot of snapping distance against target distance for toads wearing base-out prisms over both eyes. The undershooting caused by the prisms increases with their strength. ●, Minimum angular deviation caused by each prism is 2.5°. ○, Minimum angular deviation is 9°. ▲, Minimum angular deviation is 15°. The solid lines show the predicted snapping distance on the assumption that toads employ stereopsis alone. In these predictions the distance (about 1 cm) between the prism and the eye is taken into account. Data from four toads snapping at a yellow cylinder moved by hand in their frontal visual field. *b*, Relation between *S* and a prediction of *S* derived from the expression $0.94B + 0.06M + 1.1$, with *M* and *B* defined as in Fig. 2*b*. This expression indicates that when targets are viewed binocularly disparity provides the major cue to distance, accommodative information playing a relatively minor role. The predicted value of *S* has been calculated for each data point (from plots like that of Fig. 3*a*) in which the target distance is 15 cm or greater. ●, Snapping responses of toads wearing one of five different prism strengths ($\delta = 2.5^\circ, 6^\circ, 9^\circ, 12^\circ, 15^\circ$). ○, Data points from toads wearing 136 mm lenses and one of three prism strengths (2.5°, 6°, 9°). The lines above the scattergram indicate the range of data points covered by each prism or lens combination. Data from five toads. The goodness of fit of the data to this expression over the whole plot shows that the expression is an acceptable summary of the results.

convergent eye movements, a much greater range of disparities (about 35°) must be measured than is the case in human vision where a relatively narrow but exceedingly sensitive band of disparity detectors can be shifted to any desired position in depth. To confirm that eye movements are not essential for stereopsis in toads the effect of base-out prisms on snapping distance was examined in one toad with its medial and lateral extra-ocular muscles sectioned, and, as expected, base-out prisms continued to cause under-shooting.

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Functional test of tight junctions in the mouse blastocyst

THE mouse blastocyst contains a fluid-filled cavity, the blastocoele, which in normal Q-strain embryos first appears at about 83 h post coitum, when cleavage has produced a ball of about 30 cells¹. Two factors could be involved in the formation of the blastocoele: the secretion of fluid, which seems to be brought about by the coalescence and subsequent release of fluid-filled cytoplasmic vacuoles², and the development of tight intercellular junctions. Such junctions are first observed at the eight-cell stage, around the periphery of the embryo³; in the blastocyst they have increased in number and complexity to form the so-called 'zonula occludens', a permeability seal that can exclude lanthanum tracer from the blastocoele and thus presumably plays a role in maintaining the fluid turgor of the blastocyst⁴. By 'immunosurgical' dissection⁵ of Q embryos, we have investigated the stage of normal development at which the permeability seal becomes functional. We report here that impermeability to antiserum was not achieved until the mid-blastocyst stage (45–50 cells), and was not hastened or delayed by experimentally increasing or decreasing total cell number.

The technique of immunosurgery involves incubating embryos in the presence of anti-mouse antiserum; after thorough washing, the embryos are then incubated in complement. Cells coated with antiserum are briskly lysed; interior cells survive if and only if the antiserum has been denied access to them. As well as embryos grown *in vivo*, some aggregated and control cultured embryos were studied, to determine whether the total number of cells in the embryo affects the stage at which antiserum is first excluded. Table 1 shows that antiserum treatment lysed all cells in morulae and early blastocysts, up to 96 h after ovulation. At 97 h, 9% of *in vivo* blastocysts yielded some surviving cells; at 106 h, by which time the embryos were late blastocysts (containing over 100 cells on average), non-lysed cells were recovered from all.

Geometrical considerations suggest that a 17-cell embryo

Table 1 Isolation of inner cell masses from blastocysts of different ages by antiserum treatment

Source	Age (h post coitum)	Estimated mean cell number of embryos†	No. of embryos subjected to immunosurgery	% Embryos with cells surviving	No. of surviving cells Mean‡	Range
<i>In vivo</i>	90	45.1*	25	0.0	—	—
	93	52.2*	10	0.0	—	—
	95	57.7*	13	0.0	—	—
	96	60.7*	23	0.0	—	—
	97	67.2(6)	58	8.6	15.0 (2)	15, 15
	106	109.3(7)	33	100.0	35.4 (10)	22–62
<i>In vitro</i> (single)	97	44.6*	27	0.0	—	—
	105	63.4*	71	14.1	21.8 (9)	18–25
	106	66.4*	37	27.0	24.0 (3)	21–27
	110	83.5(4)	38	50.0	20.1 (8)	15–26
	114	94.6*	18	72.2	22.0 (8)	15–28
<i>In vitro</i> (double)	97	—	17	0.0	—	—
	105	—	10	20.0	39.5 (2)	37, 42
	106	—	31	22.6	41.0 (7)	27–57
	112	—	8	75.0	42.6 (3)	38–47
	114	—	23	91.3	37.5 (6)	29–60
<i>In vitro</i> (triple)	114	—	13	100.0	58.3 (7)	48–78

*Estimates of cell number taken from Fig. 2, ref. 1.

†Numbers in parentheses represent the number of observations on which the mean is based.

Spontaneously ovulating females of the randomly bred Q- strain were used, kept in controlled lighting conditions with 16 h light, and 8 h dark centred on either 2400 or 1400. Ovulation and mating were assumed to have occurred at the midpoint of the dark period preceding the finding of the copulation plug. Embryos were either flushed from the uterus on day 4 or 5 of pregnancy (*in vivo*), and the zona pellucida removed with pronase, or recovered from the oviduct on day 3 of pregnancy and grown *in vitro* to a comparable stage of development. Cultures were carried out at 36 °C in a gas-phase of 5% CO₂ in air, under paraffin oil in 20 µl drops of medium 16 (ref. 14) after removal of the zona pellucida. Some of the zona-free eight-cell stages were aggregated into pairs or triplets before culture¹⁵. The method of antiserum treatment was similar to that used by Solter and Knowles⁷. Rabbit antiserum raised to mouse spleen cells was used at a dilution of 1/30 in phosphate-buffered medium¹⁴. Zona-free embryos were incubated in antiserum for 30 min at 36 °C, washed in phosphate-buffered medium at least four times, then incubated in 10% guinea pig complement (Wellcome, lyophilised) in isotonic saline for 30 min at 36 °C. Gentle pipetting dissipated the lysed and dead cells, so that any surviving interior cells could be clearly seen. These were counted after air-drying¹⁶ and staining with lactic-acetic orcein. Cell counts were carried out by the same method on some embryos before antiserum treatment.

should have at least one interior cell⁶. Direct observations have shown that cells may in fact become completely surrounded as early as the eight-cell stage⁷. If peripheral tight junctions formed a fully effective seal when they first appeared, at the eight-cell stage, immunosurgery should allow the isolation of even a single interior cell. It turns out that not only do no morulae survive antiserum treatment, but that the smallest number of cells that can be isolated in this way is about 15 (Table 1). Fifteen interior cells correspond to a blastocyst comprising *in toto* 45–50 cells^{8–10}. By this stage, the blastocyst *in vitro* is known to undergo a series of relatively rapid contractions of its cavity, followed by slower expansions¹¹. The blastocoele fluid seems to be restored and maintained by the action of the trophecto-derm^{12–13}, desmosomes between trophecto-derm cells are seen for the first time⁴, and the apparent turgor of the expanded blastocyst confirms that a permeability seal is operative. On the other hand in normal development the blastocoele first appears at about the 30-cell stage, before the tight junctions are fully capable of excluding antiserum. Unless the junctions form a one-way seal, impermeable to the passage of fluid from the inside outwards but not yet from the outside inwards, the existence of a fluid-filled cavity at this stage must depend on factors other than a permeability barrier. For example, the cavity could be maintained by a continuous release of fluid from the cytoplasm, or merely by a change in shape of the peripheral cells.

The results for aggregated embryos and their cultured controls are also shown in Table 1. Development *in vitro* was delayed by 7–10 h. Surviving cells were absent at 97 h, and were first observed 105 h after ovulation, both in the single and in the aggregated double blastocysts. By 114 h, non-lysed cells were recovered from the great majority of cultured embryos. The rate at which the proportion of embryos with some cells surviving increased did not differ

significantly between the single and double group, although the number of cells that survived was greater by almost twofold in the doubles than in the singles, and by almost threefold in the triples. The smallest number of cells recovered after antiserum treatment was 15 for single embryos *in vitro* (the same as *in vivo*), 27 for double, and 48 for triple embryos.

Thus, the time at which peripheral tight junctions are capable of excluding antiserum from the interior of the embryo does not depend on the size of the total cell mass. The time of first appearance of the blastocoele was also found to be independent of cell number, and was associated neither with the number of cell divisions nor with the chronological time elapsed since fertilisation¹. For blastocoele formation, the developmental clock could involve the number of DNA or chromosome replications, or possibly changes in nucleo-cytoplasmic ratio. Once blastocoele formation has begun, it may be that fluid pressure from the interior of the embryo is instrumental in the transformation of the semi-permeable system of tight junctions into a true zonula occludens.

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Splenic T-killer cells can be generated by allogeneic thymic cells in conjunction with assisting factor

CELL-MEDIATED lympholysis (CML) is known to be brought about by thymus-derived (T) killer cells that have acquired reactivity towards antigens borne on target cells. To generate such T killer cells, cooperation between two subsets of T cells within the effector population is required—namely, T helper and T pre-killer cells^{1–3}. Helper T cells are thought to recognise Ia antigens^{4,5} and/or unique H-2 antigens⁶, while T pre-killer cells are known to recognise H-2 antigens^{4,5}. Ia antigens are found on B cells as well as on a certain sub-population of T cells⁷, whereas H-2 antigens have been described on virtually all B and T cells⁷. Plate has shown that a soluble factor from alloantigen-activated T helper cells assisted T pre-killer cells to differentiate into killer cells⁸. In this instance, soluble helper factor was generated in separate cultures containing mixtures of allogeneic cells, which was then assayed in a CML system from which T helper cells had been serologically removed. Since most thymic cells are known to lack Ia antigens⁷, they are conceded to be poor stimulators in both CML and mixed lymphocyte reactions^{9–11}. Nevertheless, they possess H-2 antigens and we reasoned that they might still serve to stimulate the generation of killer cells provided an assisting factor was incorporated from an exogenous source. In this way, T killer cells might be generated without at all disturbing the effector cell population by serological deletions. The data presented here support the idea that thymocytes alone are incapable of activating T helper cells, but that in the presence of exogenously produced assisting factor they do trigger development of T killer cells. The data also indicate a simple way by which assisting factors may be studied and by which requirements for CML may be delineated.

To illustrate the simplicity by which killer cells may be generated using this method, C57BL/6 spleen cells (10^7) were co-cultured, for sensitisation purposes, with allogeneic stimulator cells (10^7 , CBA/H X-irradiated with 2,000 rad) derived from spleen and thymus. Supernatant fluids were added to certain of the cultures containing thymic cells as stimulators, to assist the generation of effector cells. These fluids were added to cultures at the outset of the sensitisation phase in concentrations that ranged from 40 to 50% of the total volume, as noted in the tables. These assisting supernatant fluids were produced by co-culturing 10^7 C57BL/6 spleen cells for 48 h in a 5% CO₂ environment with a similar number of CBA/H spleen cells that had been X-irradiated with 2,000 rad. Such cultures were centrifuged at 170g for 10 min and filtered (0.45 µm Millipore filter) to eliminate viable cells. A control supernatant fluid was produced by culturing 10^7 C57BL/6 spleen cells alone. To test for killer activity, the ⁵¹Cr release assay was performed in microtitre plates according to the method of Simpson².

CBA/H splenic cells served quite well to stimulate C57 splenic cells to become killers, whereas CBA/H thymic cells did not (Table 1). However, if supernatant fluid from allogeneic mixtures of splenic cells was included in the medium during the sensitisation phase, thymocytes were capable of sensitising the C57 splenic cells to become killers. Killer cells produced were specific only for the sensitising cells (H-2^b) (see Table 1). Supernatant fluids taken from C57 splenic

Table 1 Allogeneic thymic cells serve as stimulators of killer cells provided they are assisted by humoral factor(s)

Sensitisation of C57BL/6 effector spleen cells by CBA stimulator cells* from:	Addition of supernatant fluids† derived from splenic cells	Specific lysis‡ (% ± s.d.) Effector: Target 2:1 4:1
Spleen	—	41.8 ± 9.8 52.4 ± 10.1
Thymus	—	9.6 ± 7.5 14.0 ± 5.6
Thymus	C57BL/6 + CBA/H*	40.9 ± 15.0 54.3 ± 16.7
Thymus	C57BL/6	8.6§ 11.8§

||After 5 d in culture, viable cells were counted by the trypan blue exclusion method and used as effector cells. Target cells were CBA/H splenic cells that had undergone blastogenesis for 2 or 3 d in the presence of concanavalin A (Miles) and that had been labelled with ⁵¹Cr (3 × 10⁷ blast cells incubated with 100 µCi of sodium chromate for 90 min.). ⁵¹Cr-labelled target cells (10⁵) were mixed with 2 × 10⁵ or 4 × 10⁵ effector cells, centrifuged for 5 min at 45 g and incubated for 3 h at 37 °C. Thereafter, the plates were centrifuged for 10 min at 170 µg and 100 µl of supernatant fluid was removed and assayed for released label with a gamma counter. To control for specificity of lysis, C57 effector spleen cells were co-cultured separately with splenic and thymic cells of (C57BL/6 × CBA)F₁ mice. Some of the latter were in the presence of KAF. After 5 d, effector cells sensitised to splenic cells achieved lysis equal to 52.1% and 0.7% against CBA (H-2^k) and C57BL/6 (H-2^b) target cells, respectively. Those effectors sensitised to thymic cells alone gave 10.8 and —2.0%; those sensitised to thymic cells in the presence of KAF achieved lysis of 59.1 and 6.1% respectively. In addition, those effector cells sensitised with CBA spleen failed to significantly lyse DBA/2 targets (H-2^d) (8.9%) as did effector cells sensitised with CBA thymus cells in the presence of KAF (8.0%).

*Exposed to 2,000 rad X-ray irradiation.

†Added at 40–50% of total volume of the cultures.

‡% specific lysis, [(sensitised cells—non sensitised cells/maximum release—spontaneous release)] × 100.

For these five experiments, the average maximum release was 5,079 c.p.m. and the average spontaneous release was 1,134 c.p.m.

§No s.d. provided as these values are averages of two experiments.

cells cultured alone were not able to help in generating killer cells (Table 1). The inability of C57 spleen cells cultured alone to produce killer assisting factor (KAF) could not be attributable to a reduction in cell viability at the end of the culturing interval (data not shown). An additional control not shown in Table 1 consisted of using no stimulator cells but only assisting supernatant fluid in the sensitising phase. This condition produced no killer cells indicating that both stimulator cells and KAF are essential to the process and that cellular fragments or antigens present in the supernatant fluids were not responsible for generating killer cells.

Table 2 Killer assisting factor (KAF) acts on T cells

Characterisation of Effector cells (C57BL/6)	Stimulator cells* (CBA/H)	Assisting supernatant fluid† added	Specific lysis (%) Effector: Target‡ 4:1
Whole spleen	Whole spleen	0	61.4
	Thymus	0	10.6
	Thymus	+	71.8
Splenic T cells (Nylon wool non-adherent)	Whole spleen	0	67.0
	Thymus	0	4.8
	Thymus	+	57.6
Splenic B cells (Anti-Thy-1.2 + C')	Whole spleen	0	1.0
	Thymus	0	n.d.
	Thymus	+	1.8
Whole spleen (Normal mouse serum + C')	Whole spleen	0	67.4
	Thymus	0	22.2
	Thymus	+	76.9

*Exposed to 2,000 rad X-ray irradiation

†From C57 spleen + CBA/H spleen (2,000 rad) added at 40% of the volume of the culture.

‡The maximum and spontaneous releases for this experiment were 3,893 c.p.m. and 544 c.p.m. respectively.

n.d., not determined.

Table 3 T Cells are a source of KAF

Production of supernatants	Sensitisation phase		Effector phase
Subpopulation of C57 spleen cells used to produce KAF§	C57 spleen cells sensitised by CBA cells* from	KAF supernate added†	Specific lysis (%) Effector: Target‡ 4:1
—	Spleen	—	64.8
—	Thymus	—	12.6
Whole spleen	Thymus	+	57.8
Nylon wool non-adherent (T)	Thymus	+	60.3
Anti-Ig+C' (T)	Thymus	+	45.4
Normal goat serum+C' (whole spleen)	Thymus	+	57.5
Nylon wool adherent (B)	Thymus	+	11.4
Anti-Thy-1.2+C' (B)	Thymus	+	17.8
Normal mouse serum+C' (whole spleen)	Thymus	+	50.7

*Exposed to 2,000 rad X-irradiation.

†50% of total culture volume.

‡Maximum and spontaneous releases were 5,132 c.p.m. and 927 c.p.m. respectively.

§C57 splenic cells were separated into two fractions by passage through nylon wool columns. Other aliquots of C57 spleen cells (40×10^6) were treated with θ -specific anti-serum (Thy-1.2 as in text) plus complement (agarose absorbed guinea pig serum) or with Ig-specific anti-serum (Cappel Labs.) plus complement for 1 h at 37 °C. Some C57 spleen cells were incubated with normal mouse serum plus complement and normal goat serum plus complement as controls. Following these treatments, the remaining viable cells were mixed with X-rayed CBA/H spleen cells to make assisting supernatant fluids, as described in the text.

To determine that T cells were, indeed, the killer cells in this system and to learn whether KAF was acting on killer cell precursors, fractionated splenic T cells and B cells were used separately as the intended effector cells during the sensitisation phase. Fractionated splenic T and B cell populations were obtained using nylon wool columns according to the method of Julius *et al.*¹² Since the nylon wool adherent cell population is known to consist of a mixture of B and some few T cells, this fraction was further treated with anti-Thy 1.2 serum (AKR-anti-C3H at 1:10 final dilution) plus agarose-absorbed guinea pig serum. The results shown in Table 2, which represent one of two parallel experiments, substantiate the known fact that splenic T cells, when confronted with allogeneic spleen cells as stimulators, will kill allogeneic target cells to approximately the same degree as do whole spleen cells acting as effectors (67.0% compared with 61.4%). Furthermore, just as with whole spleen cell populations, splenic T cells did not become effector cells when co-cultured in the presence of allogeneic thymic cells alone (10.6% and 4.8%). Splenic T cells, however, just as whole spleen cells, developed killer T cells when provided the double stimuli of allogeneic thymic cells and KAF (57.6% and 71.8%). Splenic B cells were unable to generate effector cells either in the presence of allogeneic whole spleen cell stimulators (1%) or in the presence of thymic cells and KAF (1.8%). As control, whole spleen cells intended as effectors were exposed to normal mouse serum plus complement prior to sensitisation. That these cells showed a slight increase in their ability to lyse target cells may be attributable to experimental error although other possibilities exist.

Experiments were also conducted to identify the cell source of KAF. This was accomplished by nylon wool separation of whole spleen cell populations and by serologically eliminating sub-populations from spleen cells and using the remainder in conjunction with irradiated allogeneic spleen cells for supernatant fluid production.

When splenic cells were depleted of B cells either with nylon or by anti-Ig serum and complement, the remaining cells produced KAF (Table 3). On the other hand, deleting T cells from splenic populations with nylon wool or serologically, eliminated KAF production. Indeed, these data (lines 7 and 8, Table 3) also show that KAF does not originate from the X-irradiated stimulator cells. Thus, two separate lines of evidence identify the source of KAF as T cells. We do not, however, exclude the possibility that the factor might be produced by other cells under other conditions.

Thus, a new simplified method has been described by

which thymic cells in conjunction with exogenously produced assisting factors will generate killer T cells for CML. KAF has been shown to be produced by T cells that are stimulated by allogeneic spleen cells. KAF was found to act upon T cells during the sensitising phase. Thymic cells alone serving as stimulators in the sensitisation phase, did not generate T killer cells. Exogenous KAF is therefore required as an additional signal when thymic cells are used as stimulators of pre-killer T cells. Whether KAF can be produced by stimulating T helper cells with cells that are not allogeneic, or with mitogens (concanavalin A) is not known. The exact requirements for the generation of KAF in this system as well as its antigenic and strain specificities are currently being investigated.

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Cytotoxic T cells kill influenza virus infected cells but do not distinguish between serologically distinct type A viruses

THE molecular basis of recognition and target cell killing by cytotoxic T cells is still unknown. Doherty and Zinkernagel showed that mouse cells infected with lymphocytic choriomeningitis virus can be lysed by immune cytotoxic T cells but only if both cell types share at least part of the major

histocompatibility (H-2) region (see ref. 1). H-2 compatibility is also required for cell killing in other viral systems²⁻⁴, and for cells carrying non-viral antigens⁵⁻⁷. Our aim is to analyse the virus specificity of cytotoxic T cells, and the nature of their antigen recognition units. This requires a virus system with a small number of well characterised protein components, amenable to biochemical and genetic manipulation, as well as cytotoxic cells that will lyse target cells in a short term assay to minimise nonspecific cytotoxicity. Influenza virus offers many advantages for this type of study. The virion contains only two well characterised surfaced antigens, haemagglutinin (H) and neuraminidase (N), that are also expressed on the surface of infected cells⁸⁻¹¹, and can be purified in quantity. Virus strains are available with serologically distinct surface proteins, and with different internal proteins^{12,13}. Here we describe the generation of potent cytotoxic cells that are specific for histocompatible influenza virus-infected cells, but exhibit extensive cross-reactivity between the different type A influenza virus strains.

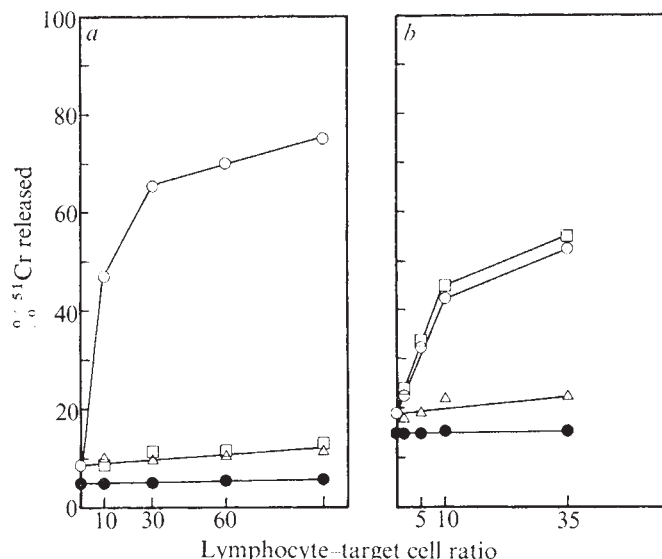


Fig. 1 Cytotoxic T cell-killing of H-2 compatible target cells infected with type A/Jap/Bel influenza virus. ⁵¹Cr release assay was carried out in microtitre II plates⁶, using 2×10^4 lymphoblasts or 10^4 P815 cells and varying numbers of immune cells per well as described by Simpson *et al.*²². Incubation was for 3 h. %⁵¹Cr release = (counts released by target cells/counts released by 2.5% Triton X-100) $\times$ 100. Induction of cytotoxic cells: spleen cells after removal of red cells by 0.184 M NH_4Cl , were cultured with 2×10^6 stimulator cells for 5 days at 37 °C in Sterilin vials in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), 5×10^{-5} M mercaptoethanol (ME), and antibiotics (4×10^6 spleen cells in 4 ml per vial). Stimulator cells were syngeneic macrophages infected with type A/Jap/Bel (10^7 peptone-induced peritoneal cells were incubated with 100 HA units type A/Jap/Bel in siliconised tubes for 90 min at 37 °C and washed). Infection of target cells: 10^7 P815 mastocytoma cells (H-2^d, DBA/2) in 1 ml RPMI 1640 were incubated with 100 μCi ⁵¹Cr and 100 HA units type A/Jap/Bel for 90 min at 37 °C, washed three times, and incubated at 10^6 cells per ml in RPMI 1640 + 10% FCS for another 4 h. Lymphoblasts were obtained by stimulating 10^7 spleen cells in 10 ml RPMI 1640 medium supplemented with 10% FCS, 5×10^{-5} M ME and antibiotics for 2 d with 10 μg *E. coli* lipopolysaccharide in 25 ml Falcon flasks. Large blast cells were purified on a BSA gradient²¹ and labelled and infected as above. *a*, BALB/c spleen cells, cultured with macrophages for 5 d and assayed on ⁵¹Cr-P815 target cells. (Δ), Normal (unprimed) spleen cells, stimulated with infected macrophages, assayed on infected target cells; spleen cells from intranasally primed mice cultured with infected macrophages ($\circ$), or cultured with uninfected macrophages ($\square$), assayed on infected target cells; ($\bullet$) as ($\circ$), but assayed on uninfected target cells. *b*, CBA/H spleen cells (from intranasally primed mice) were cultured with infected macrophages and assayed on ⁵¹Cr-CBA/H lymphoblasts. ($\circ$) infected target cells; ($\bullet$) uninfected target cells; (Δ) cytotoxic cells treated with AKR anti-Thy 1.2²³ + C', assayed on infected target cells; ($\square$) cytotoxic cells treated with normal AKR serum and C', assayed on infected target cells.

An *in vitro* system for the secondary induction of spleen cells from primed mice¹⁴⁻¹⁸ was used to generate cytotoxic T cells which lysed influenza virus-infected cells in a 3-h assay. Virus was grown in embryonated chicken eggs and allantoic fluid was used as inoculum. Mice (2-3 months old) were primed by infecting them intranasally on two consecutive days with 4 HA units (0.05 ml) of influenza virus strain A/Jap/Bel (WHO nomenclature¹⁹); 5-12 weeks later spleen cells were put into culture with infected syngeneic macrophages for the *in vitro* generation of cytotoxic cells. After 5 d of culture, the cells were assayed for their ability to lyse ⁵¹Cr-labelled target cells infected with different strains of influenza virus. Either histocompatible P815 mastocytoma cells (H-2^d, of DBA/2 origin) or syngeneic lymphoblasts activated by *Escherichia coli* lipopolysaccharide (LPS) served as target cells. The latter were purified on bovine serum albumin gradients²¹, since virus replication occurred in blast cells only. We tested a number of mouse strains, but report only results with cytotoxic cells from CBA/H (H-2^k) or BALB/c (H-2^d) mice.

Primed BALB/c spleen cells cultured for 5 d with infected syngeneic macrophages yielded cytotoxic cells which lysed P815 cells infected with type A/Jap/Bel, but not uninfected P815 cells (Fig. 1a). Similarly, primed CBA/H spleen cells cultured with syngeneic type A/Jap/Bel infected macrophages killed infected lymphoblasts, but not uninfected cells (Fig. 1b). Over a number of experiments the spontaneous release of ⁵¹Cr from infected P815 tumour cells was lower (5-15%) than that from infected lymphoblasts (15-30%); and the latter cells showed lower specific maximum ⁵¹Cr release. Lymphoblasts, however, have the considerable advantage of permitting studies of humans and outbred animals. The *in vitro* generation of the virus-specific cytotoxic cells requires the priming of spleen donors and the presence of virus-infected macrophages (Fig. 1a). The virus-specific cytotoxic cells are thymus-derived cells, since treatment with AKR antiserum to Thy 1.2 and complement²³ abolished the cytotoxic activity of CBA/H cells, whereas normal AKR serum and complement had a negligible

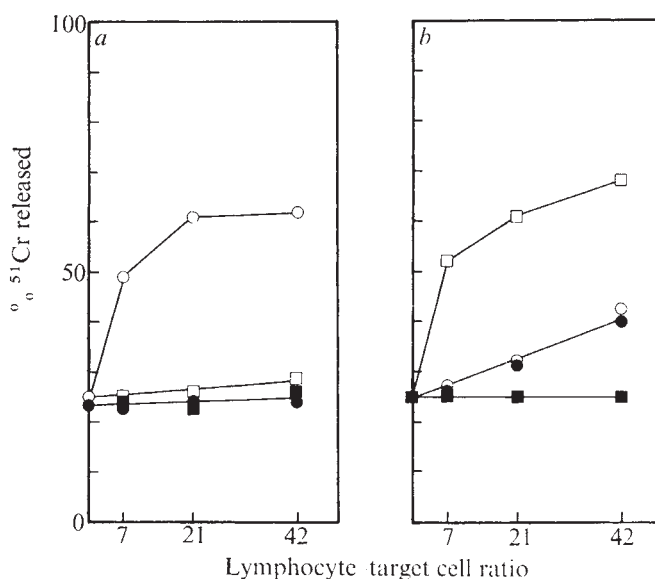


Fig. 2 H-2 restriction of cytotoxic killing. Experimental details, as for Fig. 1. BALB/c and CBA/H cytotoxic cells (CT) were generated *in vitro* by incubating spleen cells from mice that were primed with type A/Jap/Bel with syngeneic macrophages infected with type A/Jap/Bel. Syngeneic or allogeneic ⁵¹Cr-labelled lymphoblasts (LBL) were used as target cells. *a*, BALB/c CT cells assayed on infected BALB/c LBL ($\circ$), or uninfected BALB/c LBL ($\bullet$); CBA/H cytotoxic cells assayed on infected BALB/c LBL ($\square$), or uninfected BALB/c LBL ($\blacksquare$). *b*, BALB/c CT cells assayed on infected CBA/H LBL ($\circ$), or uninfected CBA/H LBL ($\bullet$); CBA/H CT cells assayed on CBA/H infected LBL ($\square$), or CBA/H uninfected LBL ($\blacksquare$).

effect (Fig. 1b). Similar results were obtained when BALB/c cells were treated with anti-Thy 1.2 (not shown).

The killing of influenza virus cells showed H-2 restriction similar to that observed with other viral systems¹⁻⁴. BALB/c (H-2^k) cytotoxic cells killed infected syngeneic BALB/c lymphoblasts but not infected allogeneic CBA/H (H-2^k) lymphoblasts (Fig. 2). Conversely, CBA/H cytotoxic cells killed infected CBA/H but not BALB/c lymphoblasts. A low level of cytotoxic activity was observed by the BALB/c lymphoblasts against allogeneic CBA/H lymphoblasts, but this cytotoxicity was directed equally to uninfected as well as infected cells.

The specificity of recognition by the virus-specific cytotoxic T cells was assayed using H-2 compatible target cells that were infected either with heterologous strains of type A influenza virus A/Jap (H₂N₂), A/Bel (H₃N₁), A/X31 (H₃N₂) (ref. 20) or with a B strain of influenza virus (B/Hong Kong). The type A viruses share internal antigens with type A/Jap/Bel (H₂N₁) but contain either serologically distinct haemagglutinins (H₂ or H₃) and/or neuraminidases (N₂). Note that type A/X31 does not share any surface antigens with type A/Jap/Bel, and that types A/Jap and A/Bel share the haemagglutinin or neuraminidase respectively. The type B/Hong Kong differs from the type A strains in both internal and surface antigens. BALB/c cytotoxic cells sensitised against type A/Jap/Bel lysed P815 cells infected with type A/Jap/Bel, A/Jap, A/Bel and A/X31 with equal efficiency, but did not lyse cells infected with type B/Hong Kong (Fig. 3). Conversely, incubation of spleen cells from B/Hong Kong primed mice with B/Hong Kong infected macrophages generated cells cytotoxic for B/Hong Kong infected but not A/Jap/Bel infected P815 cells (data not shown). We obtained the same specificity results when infected CBA/H lymphoblast cells served as target cells, and when cells were infected with another type B virus (B/Lee).

Our results demonstrate that T cell-mediated cytotoxicity of cells infected with influenza virus shows H-2 restriction as previously described for other virus systems¹⁻⁴. Of special

interest is the finding that at least a major proportion of the cytotoxic T cells are unable to discriminate between different type A influenza virus strains, whereas humoral antibodies can readily distinguish the haemagglutinin and neuraminidase proteins of these type A viruses. The T cell killing is virus-specific, since cells infected with type B influenza virus are not recognised by the cytotoxic cells generated with type A virus. Our results in the secondary response differ from those of Cambridge *et al.*²⁴, who report that a primary cytotoxic response is specific for the viral haemagglutinin in an 18-h assay. During the preparation of this manuscript we learned the Doherty and collaborators have observed cross reaction between type A viruses similar to our findings²⁵.

There are several possible explanations for the apparent difference in T and B cell recognition. T cells may recognise one of the shared internal viral proteins, provided it is expressed at the surface of infected cells. Alternatively, if the surface proteins (haemagglutinin and/or neuraminidase) are involved in T cell-recognition, the T cells would need to recognise a shared region of these surface proteins which for some reason is not immunodominant for B cells. Viral proteins expressed at the surface of infected cells may have a different conformation from those expressed at the surface of virions, resulting in the exposure of a shared region. It is also possible that T cell-mediated cytotoxicity is a more sensitive method of detecting cross reactivity than humoral antibody assays. Further studies with purified viral proteins will enable us to differentiate between these possibilities. Whatever the molecular basis of our observations, the cross reactivity for type A influenza virus strains at the T cell level is of obvious importance in immunisation against and the immunopathology of viral infections.

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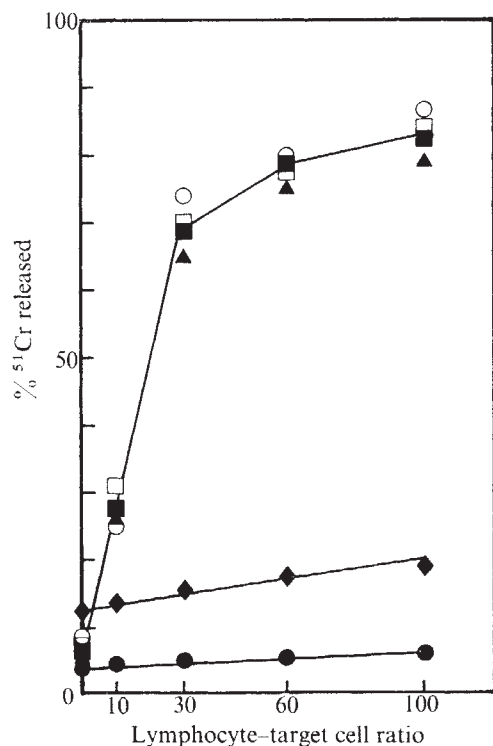


Fig. 3 Virus specificity and cytotoxic killing. Experimental details as for Fig. 1. BALB/c cytotoxic cells were generated against influenza virus type A/Jap/Bel. Target cells were ⁵¹Cr-P815 cells infected with the following strains of influenza virus: ○, A/Jap/Bel (H₂N₁); ▲, A/X31 (H₃N₂); □, A/Jap (H₂N₂); ■, A/Bel (H₃N₁); ◆, B/Hong Kong; ●, uninfected.

In vitro induction of immunological memory against testicular autoantigens

By culturing normal rodent lymphocytes together with foreign stimulator cells, lymphocytes are generated, which can destroy target cells bearing transplantation antigens identical with the ones on the stimulator cells¹⁻³. When these stimulated lymphocytes are cultured further in the absence of the primary cellular antigen, they will revert, morphologically, to small lymphocytes⁴⁻⁶. These primed 'secondary' (2°) lymphocytes⁷ are functionally oligoclonal memory cells which can be reactivated only by stimulator cells, which resemble, antigenically, the ones that caused the primary stimulation. We have studied the occurrence of a parallel phenomenon with self, rather than foreign, tissue and have established that normal rat lymph node cells can be autosensitised *in vitro* against dissociated testis cells from the same donor animal⁸. I report here that the self responsive activated lymphocytes can also be made to revert back to quiescent small secondary lymphocytes

and that immunological memory can be induced *in vitro* against autoantigens.

The *in vitro* system used is a tissue culture correlate of experimental autoimmune orchitis⁸. In mixed cultures of fresh lymphocytes and autologous, trypsin dissociated testis cells, lymphoblasts appear within 4-5 d, which can induce progressive orchitis when reinjected into syngeneic recipients⁸, and *in vitro* specifically destroy syngeneic target cells (H.W., unpublished).

Following the protocol outlined in Fig. 1, I isolated the autoreactive lymphoblasts and allowed them to revert back to small secondary lymphocytes. The cellular events taking place in secondary EAO cultures, containing secondary lymphocytes plus relevant stimulator cells, are remarkable. In such cultures, large aggregates of lymphocytes and testis cells are formed within 12 h. Within 24 h of culture, most of the small lymphocytes have been retransformed into lymphoblasts. Cultures containing secondary EAO lymphocytes together with allogeneic testis cells, however, do not develop a demonstrable lymphocyte response.

These morphological events are exactly paralleled by the ³H-thymidine uptake observed in the cultures. The data in Table 1 represent one of six consecutive experiments. They demonstrate that L.BDV secondary lymphocytes which were primed against autologous testis cells respond strongly when exposed to syngeneic L.BDV testis cells.

Table 1 MHC specific restimulation of 2° lymphocytes which were primarily autosensitised against autologous testis cells

Stimulators in 2° cultures	Responder cells	
	2° EAO-L.BDV lymphocytes	Fresh L.BDV lymphocytes
Cell type		
Testis		
L.BDV	30'956 ± 2'380	1'510 ± 257
Lewis	4'305 ± 945	2'247 ± 141
L.BDV	114'507 ± 9'306	1'834 ± 298
Lymphocytes		
Lewis	13'485 ± 2'535	6'305 ± 325

L.BDV lymphocytes were *in vitro* autosensitised against autologous trypsin dissociated testis cells. 2° lymphocytes which had been cultured for 11 d in the absence of external autoantigen were confronted in microtitre cells with syngeneic L.BDV and MHC-congenic (with different MHC, but identical genetic background) Lewis testis cells, and irradiated lymph node lymphocytes. In parallel cultures 1×10^6 fresh L.BDV lymphocytes were incubated with the four types of stimulator cells.

The same secondary lymphocytes do not react against allogeneic Lewis testis cells. These results demonstrate the immune specificity of the autosensitised secondary EAO lymphocytes. Furthermore, since L.BDV and Lewis rat strains are congenic with respect to their major histocompatibility gene complex (MHC), sharing an identical genetic background, and differ only at the MHC, these data, together with results obtained in other congenic combinations (for example, Table 2), suggest that MHC autoantigens have been recognised in the secondary reaction.

Unexpectedly, 2° EAO lymphocytes were not only re-stimulated by syngeneic testis cells, but also, to an even higher rate, by syngeneic lymph node cells (Table 1). Fibroblasts, on the other hand, did not elicit 2° responses. Two explanations may account for this phenomenon. First, lymphocytes may share cross-reacting autoantigens with testis stimulator cells. On the other hand, it is possible that the primary EAO reaction is the sum of two autoimmune components, one directed against testis cells, and the other against some cellular subpopulation among the lymph node cells. Accordingly, in the 2° reaction, the lymphocyte clones reacting with testis stimulators should be distinct from those activated by syngeneic

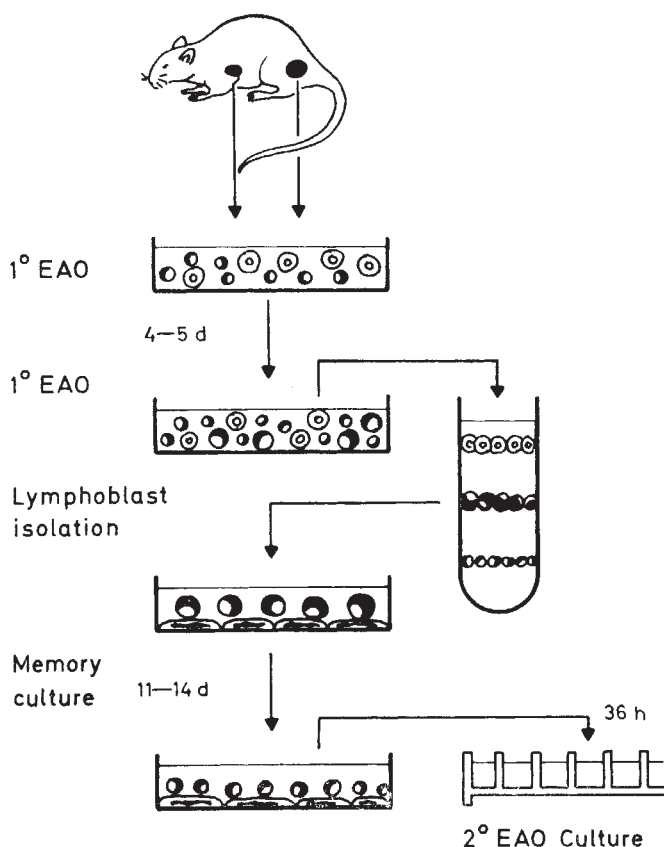


Fig. 1 *In vitro* generation of autosensitized EAO secondary cells. For primary autosensitisation 30×10^6 normal rat lymph node lymphocytes and 3×10^6 trypsin dissociated testis cells were incubated in 6 ml medium (Eagle's medium + 15% heat inactivated horse serum). After 4-5 d, the stimulated cultures were fractionated on a discontinuous Ficoll density gradient. The gradient consisted of five layers with decreasing density varying from 1.10 to 1.06 g ml⁻¹, and a top layer of Ficoll-free Eagle's medium. Centrifugation was at 10,000 r.p.m. (Beckman head SW27) for 60 min at 4°C. The purified blast fractions, containing more than 95% lymphoblasts, were seeded together with allogeneic embryonic fibroblasts (2×10^6 lymphoblasts, 1×10^6 fibroblasts) in 10 ml culture medium into 100 mm Petri dishes. After 11-14 d, the reverted small 2° lymphocytes were collected and confronted with fresh stimulator cells in microtitre plates (2×10^5 2° lymphocytes plus 1×10^6 irradiated lymph node stimulator cells (2,000 rad), or 1×10^6 dissociated testis cells, respectively). For determination of ³H-thymidine uptake, ³H-thymidine was added 24 h after starting the 2° cultures, and cells were collected in a multiple harvester after overnight incubation.

lymphocytes. Indeed, such 'spontaneous' reactions have been repeatedly described⁹⁻¹².

What is the nature of the autoantigens that elicit the secondary EAO reaction? The results shown in Table 1 suggest that antigens of the MHC are involved in the reaction. These H-1 antigens may be recognised in their native state, as 'unaltered self', by the secondary lymphocytes. On the other hand, these determinants may have been modified artificially by the culture procedures. In the latter case, the EAO reaction would correspond to lymphocyte reactions directed against syngeneic stimulator cells that have been altered either chemically¹³, by virus infection¹⁴ or by tumour transformation¹⁵.

The primary EAO reaction observed was directed against syngeneic testis cells which were dissociated by trypsin digestion. This treatment may either change normal surface antigens present on the surface membranes, or expose normally hidden determinants¹⁶. The finding that 2° cells primed with trypsin-digested testis cells can react optimally with syngeneic stimulator lymphocytes which have not been chemically treated at any time point, argues against this possibility. Moreover, a 2° reaction can also be readily initiated by testis cells that were mechanically and not enzymically dissociated.

Table 2 Lack of effect of heterologous culture sera on the specificity of the 2° reaction.

Stimulator cells	Serum additives	
	Horse serum	Foetal calf serum
L.AS2	10,157 ± 764	10,752 ± 1,164
Lewis	1,210 ± 251	1,892 ± 191

L.AS2 lymphocytes were primarily autosensitised against autologous testis cells, and reverted to 2° lymphocytes in the presence of horse serum. The washed 2° lymphocytes were tested for their immune specificity in the presence of either horse serum or foetal calf serum. The antigens in the 2° reactions were syngeneic or congenic freshly dissociated testis cells.

Another possible cause of 'self modification' may be the heterologous serum present in the culture medium. Several investigators have taken pains to show that horse serum contains components that will readily bind to mammalian cell surfaces^{17,18}. Such heterologous serum proteins could act as the foreign determinants required for an 'altered self' type of reaction. We tested this possibility by confronting 2° EAO lymphocytes (strain L.AS2), which had been cultured in horse serum medium, with syngeneic L.AS2 and MHC congenic Lewis stimulator cells either in medium containing horse serum, or, in parallel, in medium substituted with foetal calf serum. Medium supplemented with autologous serum could not be used, since this serum contains factors which specifically interfere with self recognition¹⁹. As shown in Table 2, the supplementing sera did not affect the specificity of the 2° reactions. Since it is probable that horse and foetal bovine serum proteins do not exhibit marked immunological cross-reactivity, alteration of MHC antigens by heterologous serum proteins does not seem to be a cogent explanation for the *in vitro* EAO. Moreover, it should be noted that, if foreign serum components played a critical role in the EAO reaction, the 2° lymphocytes, which possess normal amounts of MHC antigens themselves, should stimulate each other without the addition of any further target antigens.

Another possible explanation for self alteration is neo-expression of latent virus antigens on the stimulator cells. This possibility cannot be formally excluded at present and, indeed, there are claims that mouse lymphocytes can spontaneously express virus antigens after 24 h of culture²⁰. It should, however, be emphasised that in

secondary cultures the first signs of immune recognition and reaction against freshly isolated stimulator cells were noted within the first few hours of contact. This is a time span hardly sufficient for spontaneous and regular expression of virus antigens.

In conclusion, the data reported here lend further support to the notion that the normal immune system contains T lymphocytes, which are potentially able to react against the body's own tissues²¹. Our autoimmune memory system offers the opportunity to directly investigate the clonally enriched lymphocytes that possess receptors for autoantigen, and may thus be a valuable tool for elucidating the physiological mechanisms responsible for self tolerance at the T cell level. A final implication of our system is more of clinical relevance. Since the autoimmune secondary cells generated *in vitro* have the capacity to discriminate exquisitely between self and non-self, and since this capacity is not lost by storage in liquid nitrogen (unpublished), a similar, though modified, system might be helpful for human tissue typing. A particular advantage of this system is that the results of the reaction can be read after less than 24 h.

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Role of receptor aggregation in complement-dependent inhibition of lymphocytes by high concentrations of concanavalin A

EVIDENCE from haemolytic systems suggests that complement activation by either the classical or alternative pathways requires a close approximation of critical molecules at the cell surface. At least two molecules of IgG antibody in close proximity are required for the first component of complement to be bound¹. Thus when increasing concentrations of IgG are added to erythrocytes in the presence of excess complement, haemolysis is minimal until a critical IgG concentration is reached. In the case of the alternative (properdin) pathway of complement activation, at least two molecules of C3b must be critically orientated together at the cell surface in order for properdin to bind and stabilise C3/C5 convertase². Thus erythrocytes have a simple mechanism of signal discrimination by which they can 'count' the number of molecules reacting with their surfaces. The cells only 'respond' by lysis when a critical number has been reached.

I present data which relate this mechanism of signal discrimination by erythrocytes to the mechanism by which cultured lymphocytes discriminate between low and high concentrations of the mitogenic lectin concanavalin A (con A). This in turn may be related to the mechanism by which lymphocytes discriminate between low and high concentrations of specific antigen to produce either an immune response or immunological tolerance³.

Low concentrations of con A activate cultured rat lymph-node cells (enhanced incorporation of ³H-uridine), whereas high concentrations inhibit the cells by a complement-dependent process^{4,5}. The tetravalent con A molecules are able to aggregate surface receptors for con A on murine and human lymphocytes, but a divalent derivative, succinyl-con A, does not have this property⁶. Furthermore, although both forms of con A, at low concentrations, are able to activate lymphocytes to transform and incorporate ³H-thymidine after 2–3 d of culture, they differ in their effects at high concentrations. Lymphocyte transformation as assessed with ³H-thymidine is inhibited by high concentrations of the tetravalent molecule, but this is not found with the divalent derivative (see ref. 7 for review).

These results suggested that receptor aggregation might be necessary for the complement-dependent inhibition by high concentrations of tetravalent con A. To examine this hypothesis, the effects of various concentrations of succinyl-con A and con A were compared in the presence and absence of complement (bovine calf serum preheated at 56 °C for 30 min). The culture period was only 6 h and this would be expected to reduce the possibility that the cultured cells themselves could secrete complement components in quantities sufficient to influence the results⁸. The succinyl-con A was prepared from con A (Pharmacia) by the double derivatisation procedure described by Gunther *et al.*⁹.

Figure 1 shows that in unheated serum incorporation of ³H-uridine was stimulated at low con A concentrations but inhibited at high con A concentrations. The latter inhibition was not detected in cultures containing serum preheated at 56 °C. This confirms our previous results^{4,5}. In the case of preheated serum, the dose-response curve for succinyl-con A was similar to that for con A except for a small, but consistent, shift of the ascending limb to the right. In the case of unheated serum, the profound inhibition observed at high concentrations of con A was not observed at high concentrations of succinyl-con A. At such concentrations of succinyl-con A the curve plateaued at approximately the same level as the peak response of the curve for con A in unheated serum.

If receptor aggregation were necessary for the complement-dependent inhibition by con A, then both of the dose-response curves for succinyl-con A should have been similar to the curve for con A in heated serum. The depression of the plateau in the case of succinyl-con A in unheated serum suggests that our preparations of succinyl-con A still retain some complement-dependent inhibitory activity; but, even so, the inhibition was much less than that produced by tetravalent con A in unheated serum. Since the response to lectins is quantitatively dependent on the lectin:serum concentration ratio^{5,9}, a possible explanation for the shift of the ascending limbs of the curves for succinyl-con A to the right of those for con A, is that the capacity of serum to bind succinyl-con A is greater than its capacity to bind con A. Cells may also show a reduced binding capacity for succinyl-con A (refs 10, 11).

Although slightly higher concentrations may be required, succinyl-con A has the same capacity at tetravalent con A to activate lymphocytes, but has much less capacity to produce high dose inhibition. Since succinyl-con A produces less receptor aggregation ('patching' and 'capping') than con A, this suggests that such aggregation is not necessary for lymphocyte activation^{12,13}, but is necessary

for lymphocyte inhibition dependent on complement. In apparent conflict with this view is a report that, whereas 'low' concentrations of con A encourage receptor aggregation, 'high' concentrations impede receptor mobility and prevent aggregation¹⁴. Others have found, however, that 'high' concentrations of con A aggregate the receptors both of lymphocytes¹⁵ and of Chinese hamster ovary cells¹⁶. In the latter case cell death occurred. Since the effective concentration of con A depends on the con A:serum ratio⁵, some of these discrepancies may be the result of different serum concentrations employed by different workers. For example, Edelman *et al.*⁶ compared the con A dose-dependence of changes in receptor aggregation using lymphocytes in medium containing albumin (which binds con A weakly), with the con A dose-dependence of changes in cell proliferation using lymphocytes in medium containing serum (which binds con A strongly¹⁷). A concentration of con A which would have been effectively 'low' in the cell proliferation assay would have been effectively 'high' in the aggregation assay. Another apparent discrepancy in the literature is evident from reports that high-dose inhibition by con A is reversible^{18,19}. If complement is involved then such inhibition would be expected to be irreversible. There are a

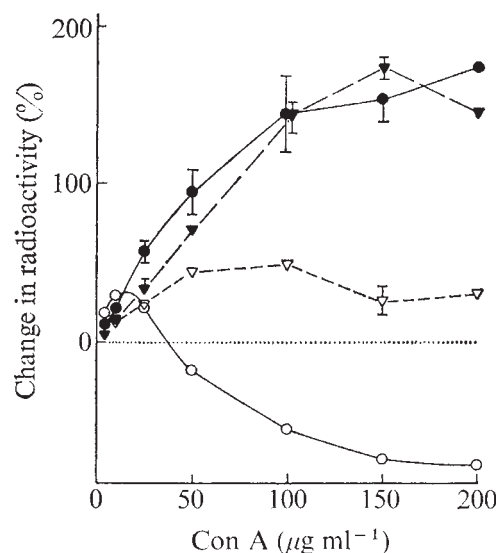


Fig. 1 Comparison of con A dose-response curves (○, ●) with succinyl-con A dose-response curves (▽, ▼) for cultures containing serum which had been preincubated for 30 min at either 38 °C (open symbols) or at 56 °C (closed symbols). Lymph-node cells (2.1×10^6) from female Wistar rats were cultured as described before⁵ in 1 ml of medium containing bovine calf serum (20%), medium 199 (80%) and 1 μ Ci of 5-³H-uridine. After 6 h at 38 °C in a humidified atmosphere of air:CO₂ (95:5), cells were harvested for the determination of acid-precipitable radioactivity. Each point is the arithmetical mean of duplicate cultures. In the case of points presented without the standard error of the mean, the limits fell within, or close to, the area covered by the point. Values (c.p.m.) for control cultures without lectin (quadruplicates) were $10,425 \pm 357$ (serum preincubated at 38 °C) and $13,066 \pm 599$ (serum preincubated at 56 °C).

number of technical explanations for the discrepancy some of which have appeared in the recent literature^{20,21}. For example Moller²⁰ has pointed out that in earlier studies¹⁸ he and his co-workers had examined the mitogen-reactivity of cells surviving preincubation for a day with high con A concentrations; the lethal effect on the non-surviving cells had been ignored. In addition, heated calf serum was used; this would have reduced the extent of 'early-onset' inhibition (see below). Recently McClain and Edelman²² carried out similar studies with preheated calf serum. They presented evidence for 'delayed-onset' inhibition (see below) appearing after a day of culture at high con A concentrations. Again, however, attention was focused on the cells

able to survive the inhibition, rather than on the inhibition itself.

The role of complement in lectin-induced lymphocyte inhibition was predicted in 1966 but experiments at that time were negative²³. The eventual demonstration of the role of complement required the use of heterologous (bovine foetal or calf) serum rather than autologous serum^{4,5}. My recent studies indicate that complement inhibitors in autologous serum prevent the onset of the complement-dependent inhibition until after the first day of culture; addition of cobra venom factor to activate the alternative (properdin) pathway can apparently by-pass these complement inhibitors and make complement components available in autologous serum to mediate the inhibition by high con A concentrations in the early hours of culture²⁴. If heterologous serum is preheated the complement-dependent inhibition is not seen in the early hours of culture (Fig. 1). However, an inhibition appears after the first day of culture⁵; we have speculated⁵ that such inhibition requires the secretion of complement components by the cultured cells⁸.

In summary, I have shown that the previously reported failure of high concentrations of succinyl-con A to inhibit lymphocytes as assessed by ³H-thymidine incorporation after 2–3 d of culture⁶ is also demonstrable during the first few hours of culture (³H-uridine incorporation). While the inhibition by tetravalent con A, as assessed with ³H-thymidine, has neither been proven nor disproven to require complement (either present in serum or secreted by cells⁸), the inhibition occurring early in culture has been shown to be complement-dependent^{4,5}. My data thus support the hypothesis that divalent succinyl-con A does not inhibit because it is unable to produce the degree of aggregation of certain molecules at the cell surface which is necessary for complement-dependent inhibition. New techniques, such as resonance energy transfer²⁵, now permit a more detailed examination of the effects of con A and its derivatives on receptor distribution. It would be of interest to correlate the results of such studies with the degree of complement-dependent inhibition at a given con A concentration.

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Use of ionophore A23187 to measure and to control free and bound cytoplasmic Mg in intact red cells

CYTOPLASMIC magnesium, whether free or complexed to nucleotides, has a fundamental physiological role as an essential cofactor for many cell enzymes, particularly those concerned with glycolysis, respiration and membrane transport. Various ingenious procedures have been applied to estimate^{1,2} or simulate^{3,4} intracellular conditions in relation to Mg but, because it is impossible to assess and control Mg²⁺ in the intact cell, most research on Mg requirements for metabolism and transport has used lysed cells⁵ and broken membrane preparations^{6,7}, which have inherent uncertainties about true concentrations and sidedness of effects. Using a divalent cation ionophore⁸ and a newly developed method^{9,10} we have investigated Mg buffering in intact human red blood cells and have found that the fresh, oxygenated, inosine-fed cell (a condition frequently used for ion transport studies^{11–14}) has three main buffer systems which bind nearly 90% of the total Mg present inside the cell in physiological conditions.

Net Mg movements across the intact red cell membrane are unmeasurably slow. Buffering was therefore explored using a selective divalent cation ionophore, A23187, in the absence of other divalent cations. Incorporation of this ionophore into the red cell membrane renders the cell highly permeable to Mg (refs 9, 10) allowing rapid equilibration of Mg ions across the membrane and with the intracellular buffers. By investigating the equilibrium distribution of Mg at different external Mg concentrations (see Fig. 1) a buffering curve can be constructed (Fig. 2). At electrochemical equilibrium the distribution of Mg ions is given by

$$\text{Mg}_i^{2+} = r^2 \text{Mg}_o^{2+} \quad (1)$$

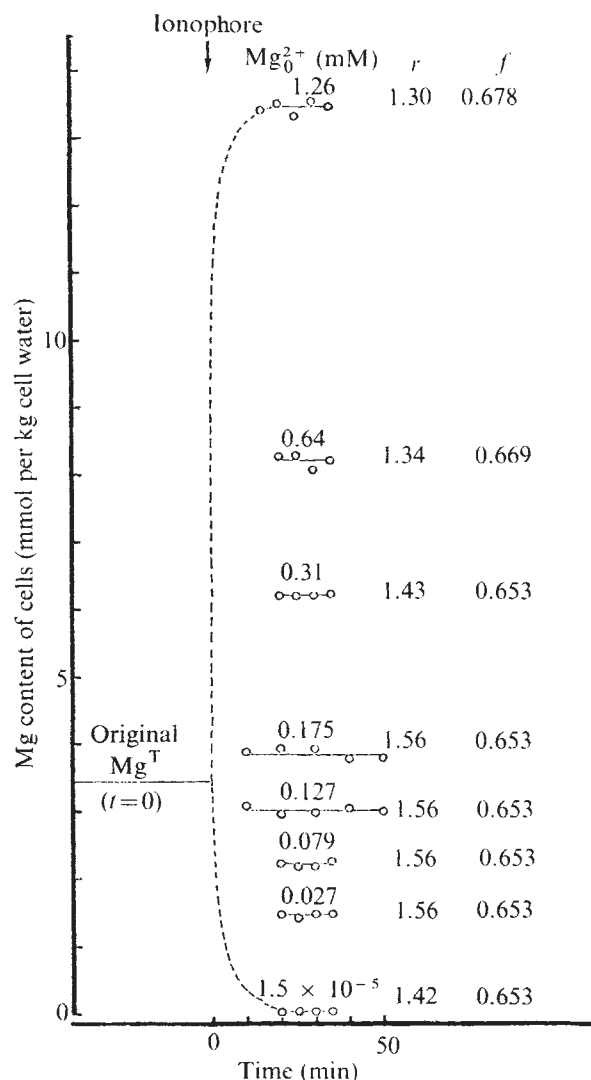
where Mg_i^{2+} and Mg_o^{2+} are the molal concentrations of ionised Mg in cell water and in the medium, respectively, and r^2 is the Donnan distribution ratio for divalent cations at equilibrium. This is related to the membrane potential (V_m) by the expression $r^2 = \exp(-2FV_m/RT)$, based on the assumption that the activity coefficients of Mg are identical on both sides of the membrane. The total Mg content of the cells is the sum of the free and bound forms

$$\text{Mg}^T = \text{Mg}_i^{2+} + \text{Mg}^B \quad (2)$$

where Mg^T and Mg^B are the molal concentrations of total and bound Mg in cell water. From equations (1) and (2) we obtain

$$\text{Mg}^B = \text{Mg}^T - r^2 \text{Mg}_o^{2+} \quad (3)$$

The total Mg content of the cells was measured by atomic absorption spectroscopy (Pye–Unicam, SP 90) in the trichloroacetic acid supernatant of cell lysates. In order to estimate Mg^T , Mg^B and r^2 , the cell water content and the membrane potential were determined at each Mg concentration, since we had found in preliminary experiments that the relatively large shifts in internal Mg concentration induced in the present experiments had an effect on cell water and on the concentration of non-diffusible anion responsible for the Donnan potential. Cell water was measured as the difference between wet and dry weights of packed-cell samples corrected for extracellular fluid using ⁶⁰Co³⁺–EDTA (ethylenediamine–NNN′N′–tetra-acetic acid) as a marker. The membrane potential was estimated from the ³⁶Cl distribution ratio¹⁵ at equilibrium, $r = \text{Cl}_o^-/\text{Cl}_i^-$,



where Cl_o^- and Cl_i^- are the molal concentrations of Cl^- in the external solutions and cell water, respectively (for details see Fig. 1).

The concentrations of internal Mg at equilibrium with different external Mg levels are shown in Fig. 1. The equilibrium distribution of Mg was unaffected by the concentration of ionophore used, indicating that the ionophore neither contributed to nor affected the buffering of Mg by the cell.

Figure 2 shows the results obtained in four different experiments where the equilibrium concentrations of bound Mg (Mg^B) obtained from Fig. 1 and equation (3) are plotted as a function of Mg_i^{2+} . Although the data are not precise enough to allow a fine distinction between individual buffers or between various similar hypothetical models, the simplest model giving a good fit requires the presence of at least three different intracellular buffer systems: (1), about 100 μ mol per kg cell water of very high affinity buffers which are already saturated with Mg when Mg_i^{2+} is about 3×10^{-8} M; (2), about 2 mmol per kg cell water of high affinity buffers, apparent dissociation constant ~ 25 –50 μ mol per kg water; and (3), a large capacity, low affinity buffer system which may bind > 1 mol Mg per mol of buffer. About 28 mmol per kg water of a buffer with an apparent dissociation constant ~ 4 mM fits the upper points reasonably well, but the fit of the lower points is improved if this component is attributed a capacity of ~ 20 mmol per kg water and two binding sites with apparent dissociation constants ~ 1 mM.

The original Mg content of the cells used in the present experiments was 3.46 ± 0.05 mmol per kg cell water. From curves such as those in Fig. 2, the Mg_i^{2+} at equilibrium with any value of Mg^T can be obtained. In this way the Mg_i^{2+} in the

Fig. 1 Mg content of cells as a function of time at different external Mg concentrations in the presence of the divalent cation ionophore A23187. Red cells from fresh blood were washed three times with about 10 vol of a solution containing NaCl, 75 mM; KCl 75 mM; Tris-Cl (pH 7.5 at 37 °C) 10 mM; and Tris-EGTA (1,2-di(aminoethoxy)ethane-*NNN'*-tetra-acetic acid) 0.1 mM and three more times with the same solution containing only 0.01 mM or no Tris-EGTA. After washes 0.25 ml of packed cells (2,000g for 5 min) were added to 2 ml of a similar medium also containing inosine (10 mM) and various concentrations of Mg and Tris-EDTA, to yield, together with Mg displaced from the cells, the final concentration of Mg_o^{2+} quoted on the figure. Before addition of ionophore, a 0.1-ml sample was taken to measure the original Mg content of the cells. At $t = 0$, 10 μ l of absolute ethanol containing 1 mg ml⁻¹ of ionophore A23187 were added to the cell suspension under vigorous magnetic stirring. At the times indicated 0.1-ml samples were withdrawn and the cells quickly separated through *n*-butyl-phthalate (BDH; density 1.042–1.045) as described previously^{9,10}, lysed in distilled water, the proteins precipitated with TCA and the Mg concentration measured in the supernatant. Water content and the ³⁶Cl distribution ratio, r , were measured as described in the text and their values are shown. The water fraction (f) is expressed as the weight of evaporated water per unit volume of packed cells. The volume of the extracellular fluid was $2.25 \pm 0.03\%$ of the volume of the cell pellet. The external concentration of Mg^{2+} at equilibrium was estimated from the nominal concentrations of Mg and EDTA in the original medium and the redistribution of intracellular Mg. The nominally Mg-free medium contained 2 mM Tris-EDTA and no added Mg; at equilibrium, Mg_o^{2+} was about 1.5×10^{-8} M and Mg_i^{2+} about 3×10^{-8} M. Variations in the external Na and K concentrations, and preincubation of the cells with inosine (variations used as part of other studies on the Mg dependence of active Na transport which will be reported elsewhere) had no effect on the Mg distribution. For clarity, only representative values for each concentration range are shown on the figure. The equilibrium concentration of intracellular Mg was measured for various lengths of time in different experiments (up to 60 min). The initial time course is only given for the extreme values of the Mg concentration range. With the ionophore concentration used in the present experiments, the half time to reach equilibrium was about 2.5 min.

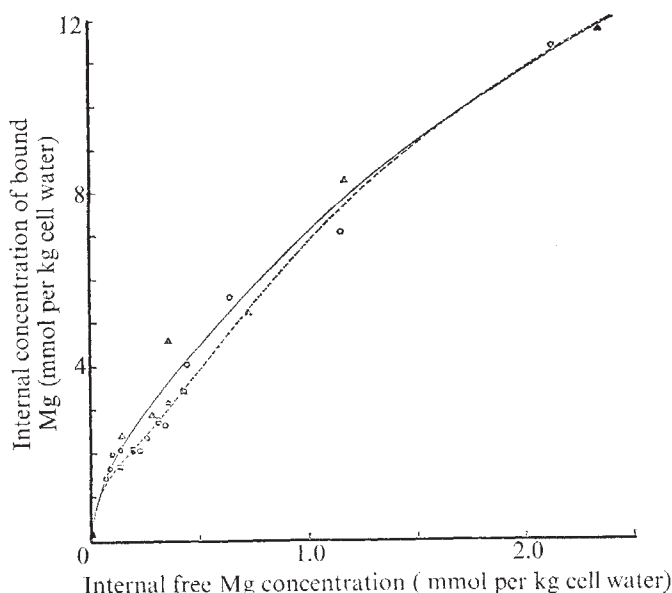


Fig. 2 Equilibrium concentration of bound Mg as a function of the free Mg concentration in cell water. Mg^B was calculated from equation (3) and using the values of Mg^T , r and f shown on Fig. 1. Different symbols correspond to different experiments. The curves correspond to the theoretical equation $Mg^B = C_0 + C_1 (Mg_i^{2+} / (K_1 + Mg_i^{2+})) + C_2 (Mg_i^{2+} / (K_2 + Mg_i^{2+}))^n$ with C and K , in mmol per kg cell water; n is dimensionless. —, $C_0 = 0.1$; $C_1 = 1.5$; $C_2 = 28$; $K_1 = 0.04$; $K_2 = 4$ and $n = 1$; ---, $C_0 = 0.1$; $C_1 = 1.8$; $C_2 = 20.5$; $K_1 = 0.03$; $K_2 = 1$ and $n = 2$.

original cells was estimated at ~ 0.4 mM which is only slightly lower than that measured with a Mg electrode in a stroma-free, freeze-thaw lysate of oxygenated red cells (0.5 mM (ref. 3)).

At present we can only speculate about the nature of the different buffers. The minute fraction of tightly bound, acid-labile Mg may be associated with cytoplasmic components or with the membrane. Analysis of membrane fragments, however, may prove misleading because this binding can be affected by the separation procedure. It is interesting to note that these binding sites cannot be accessible to Ca (refs 9, 10).

Berger *et al.*³, using simulated intracellular conditions but a much reduced haemoglobin level, estimated the dissociation constants of the complexes of Mg with ATP and 2,3-bisphosphoglycerate (DPG) as 83 μ M and 1.67 mM respectively. It is tempting to equate the low and high affinity buffers reported in this study with DPG and ATP respectively. It is difficult, however, to account for the high capacity of the low affinity buffer even in inosine-fed cells, where the levels of DPG are high^{16,17}, unless other intermediates of the glycolytic pathway or haemoglobin, which did not bind Mg *in vitro*³, are also involved. In oxygenated cells, little ATP or DPG is bound to haemoglobin so that displacement of Mg buffers from haemoglobin by high Mg concentrations would be minimal; however a progressive decrease in the internal magnesium activity coefficient at high internal Mg would have the effect of reducing the calculated amount of low affinity buffer and this possibility cannot be ruled out at present.

The method described here can be used to investigate the effect of metabolism and deoxygenation on the Mg buffer curve in intact cells as well as the Mg dependence of active Na and Ca transport.

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Transient pressure changes in Donnan systems displaced from equilibrium

PRESSURE gradients at interfaces of porous membranes can be caused by two factors: (1) discrete changes in concentrations of particles which are excluded from the aqueous pores because of their size, and (2) electrical stresses which are caused by the action of an electric field on the charged particles at the membrane-solution boundary (A.I., theoretical results to be published elsewhere). Only the first factor is important in neutral pore-containing membranes;

the second operates at the boundary of charged pores, that is, in ion exchange membranes.

One of the consequences of theoretical work (A.I., unpublished) applies to Donnan systems. A Donnan system comprises a membrane interposed between two electrolytic solutions one of which contains impermeant polyelectrolyte particles. At equilibrium, the pressure developed across the membrane equals the difference between the osmotic values of the two solutions. The latter is greater than RTC_i , where C_i is the concentration of the impermeant polyelectrolyte on one side of the membrane^{1,2}, and R and T have their usual meanings. The higher pressure develops in the system because of the increase in concentration of the impermeant polyelectrolyte particles at the membrane solution interface, brought about by the presence of an electric potential profile at that region. Solution of the Poisson-Boltzmann equations for the interfacial region (see, for example, ref. 3) shows that, at equilibrium, the concentration of the impermeant particles at the pore-solution boundary, C_i^b , is such that RTC_i^b is equal to the difference between the osmotic values of two solutions.

Since the extent of accumulation of the polyelectrolyte at the membrane-solution boundary is a function of the salt concentration in the same solution, theoretical considerations lead to the following peculiar prediction: addition of salt to the side of the membrane which contains the polyelectrolyte would cause an immediate decrease in the pressure difference between the two sides of the membrane, even though the salt addition led to an immediate increase in the difference between osmotic values of the two solutions. Thus, phenomenologically the system would behave as if the reflection coefficient for the salt were negative. The experiments described confirm that prediction.

The diffusion cell (Fig. 1) consisted of two lucite chambers held in a metal frame. The flat outer margins of each chamber were cast with a Teflon lining and the membrane between the two chambers was sealed by being pressed on

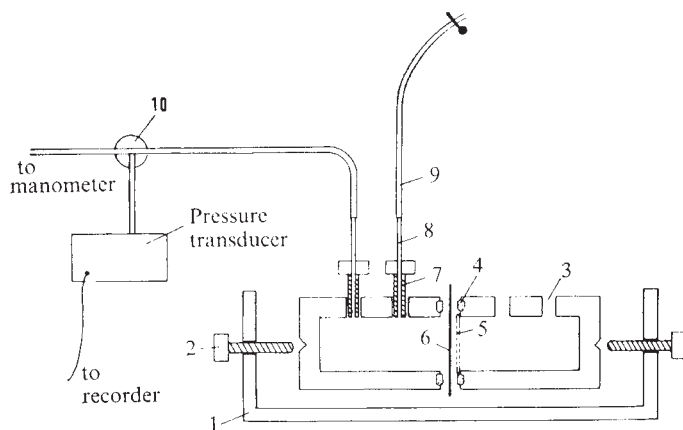


Fig. 1 The diffusion cell. 1, Metal frame; 2, fastening screw; 3, open to room pressure; 4, Teflon lining; 5, stainless steel support; 6, membrane separating the two chambers; 7, plastic screws; 8, syringe needles through the centre of the screws (7); 9, heat-shrinkable Teflon tube; 10, three-way stopcock.

its outer circumference by the Teflon O-shaped linings. On the side of the membrane with the lower pressure, a perforated stainless-steel support (0.5 mm thick) was added. Forty per cent of the support area was perforated. One chamber of the diffusion cell (left in Fig. 1) was sealed as follows: two openings in the upper part of the cell were locked by plastic screws and, to ensure hermetic sealing, the outer rim of the screws were lubricated by high-vacuum silicon grease. Through the centres of these screws, syringe

needles were tightly secured into the chamber. The other end of the needle was connected to a heat-shrinkable Teflon tube. The solution was introduced into the chamber through one of the Teflon tubes. Manipulations of the chamber ensured complete filling of the chamber with the solution. After filling, one Teflon tube was connected to the pressure transducer and the other was clamped. The other chamber (right in Fig. 1) was open to room pressure and was directly filled with the desired solution. Mixing in both chambers was effected by magnetic stirrers and the experiments were conducted at room temperature (24–26 °C). A Statham pressure transducer (Model P23AC) and a Beckman recorder (Type R dynograph) were used. The time constant of the response of the transducer to pressure changes was instantaneous compared with that of the whole diffusion cell system, which was about 3 min.

Analytical procedures included chloride analysis by titration with mercuric nitrate using phenylcarbazone as an indicator, and sodium analysis by flame photometry. Several combinations of membranes and polyelectrolyte were tried. The characteristics required of the membrane were: relatively long aqueous pores, freely permeable to small ions, but completely impermeable to the polyelectrolyte. It was important to use only symmetric membranes. Membranes in which only a thin skin was the selective barrier were obviously unsuitable for this study. The best combination that we found were porous vinyl membranes (Nalco Chemical Company, Chicago) and a sulphonated dextran, of molecular weight of about 500,000 (Sigma Chemical Company, Saint Louis). The thickness of the membranes was $\sim 100 \mu\text{m}$ and the estimated pore diameter about $60 \text{ \AA}$.

The equilibrium pressure characteristics of the system are shown in Fig. 2. The calculated osmotic values of the dextran-containing solution as a function of the chloride

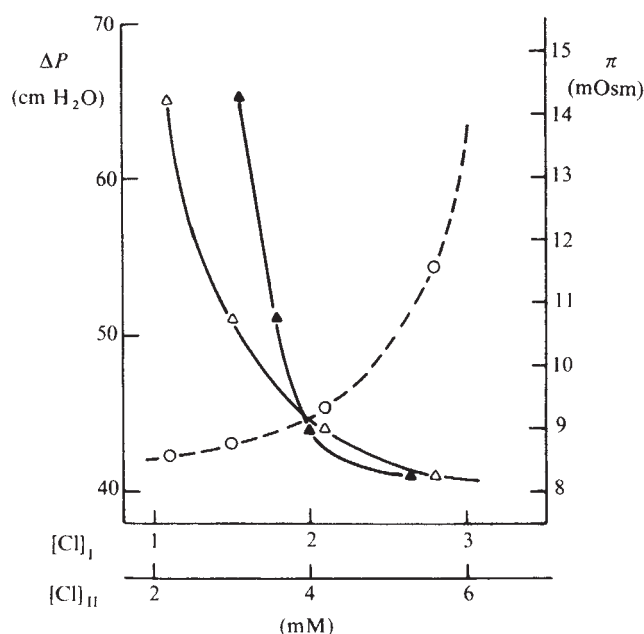


Fig. 2 The equilibrium properties of the diffusion cell when side I of the cell contained sulphonated dextran 9 mg ml^{-1} ; side II contained NaCl only. ---, Calculated osmotic value of the dextran solution as function of chloride concentration $[\text{Cl}]$. The osmotic value of side I was equal to that of side II plus $\Delta P/RT$. The osmotic value of side II was determined by multiplying the chloride concentration by 2 and by the activity coefficient of NaCl at the relevant concentration. The polyelectrolyte solution without chloride contained about 0.018 mM sulphonated dextran and 42 mM sodium. The fact that the osmotic value of the dextran solution is $\sim 8 \text{ mOsm}$ indicates that activity coefficient for Na is ~ 0.2 . Δ , $\Delta P [\text{Cl}]_I$; $\blacktriangle$, $\Delta P [\text{Cl}]_{II}$.

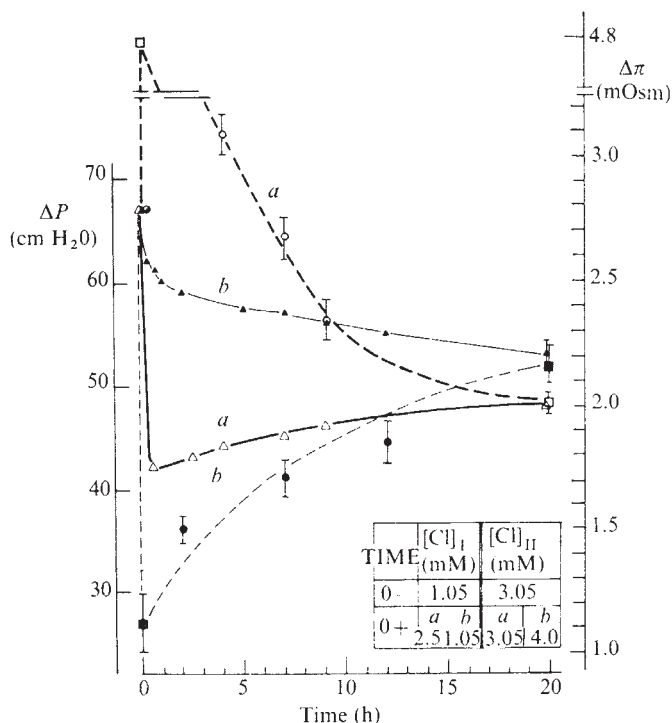


Fig. 3 Time course of change in pressure (Δ , $\blacktriangle$) in a Donnan system brought about by the addition of salt to either side of the cell. *a*, Addition to the sulphonated dextran solution; *b*, addition to the salt solution. Inset, Type of change that was effected at time zero (0_+). The initial conditions (0_-) were the same in both cases. ---, Difference in osmotic values of the two solutions as determined by the chloride concentration in both sides, $\square$, $\blacksquare$, derived by direct chloride analysis; $\circ$, $\bullet$, osmotic values based on calculations of chloride concentrations. $[\text{Cl}]_I$ was estimated from the concomitant ΔP measurements making use of the relevant curve in Fig. 2; $[\text{Cl}]_{II}$ followed from the principle of conservation of matter. The vertical lines represent upper and lower limits which resulted from uncertainties in chloride analysis ($\pm 0.1 \text{ mM}$) and in ΔP measurement ($\pm 0.5 \text{ cm H}_2\text{O}$).

content are also shown in Fig. 2. Chloride was added to the dextran solution as NaCl. The pressure changes accompanying NaCl addition to either side of the system are shown in Fig. 3.

The dashed lines in Fig. 3 represent the difference between osmotic values of the solutions on the two sides of the membrane and curves *a* show that the addition of NaCl to the polyelectrolyte solution lead to increase in the difference between the osmotic values of the two solutions and to decrease in the pressure difference across the membrane.

The results of Fig. 3 could also be accounted for by the assumption that a significant unstirred layer occurs in the side of the membrane which had the metal support. To account for the results along these lines, however, needs an assumption that Donnan equilibrium was attained instantaneously across the membrane and that the diffusion barrier was located only on the side which did not contain the sulphonated dextran. Therefore we have conducted NaCl diffusion experiments through the system with and without the perforated metal support. These experiments demonstrated that the permeability of the membrane to NaCl was 20–30% greater when the metal support was used, probably because the effective membrane area was a little more than the porous area of the metal support. At any rate, these experiments proved clearly that unstirred layers could not constitute a significant barrier in the diffusion cell. The membrane permeability to NaCl was $\sim 3 \times 10^{-4} \text{ cm s}^{-1}$. This level of permeability would correspond to unstirred layer of about $330 \mu\text{m}$ thickness. Analysis of thickness of unstirred layers as a function of stirring rates shows that

they vary from $\sim 75 \mu\text{m}$ at slow rate of stirring to $50 \mu\text{m}$ at vigorous stirring⁴. Thus, it is unlikely that an unstirred layer had a major effect in the system described here.

We suggest that these experiments support the notion (A.I., unpublished) that the pressure across the porous membrane is determined by the concentration of the impermeant polyelectrolyte particles at the membrane solution interface. The extent of accumulation of the polyelectrolyte molecules at the membrane interface is a function of the salt concentration in the solution. At equilibrium, that is, in classical Donnan systems, the accumulation of the polyelectrolyte molecules at the membrane interface gives rise to a pressure difference which is exactly equal to the difference between the osmotic values of the two solutions.

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Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment

5-AZACYTIDINE (aza-CR)—a *s*-triazine nucleoside analogue of cytidine—has strong inhibitory effects in various biological systems including neoplasms: it is a powerful bacteriostatic, antitumour and mutagenic agent¹. Aza-CR also exhibits immunosuppressive, antimitotic, radioprotective and virostatic effects¹. The cytostatic effect of aza-CR has primarily been directed against leukaemia² and it has been used to treat human solid tumours³. During a study on the morphological transformation of C3H 10T½ C18 cells by cancer chemotherapeutic agents, it was noted that elongated multinucleated cells arose in cultures exposed to aza-CR⁴. The C3H 10T½ C18 line is a clonal line of mouse embryo cells developed at the McArdle Laboratory for Cancer Research for use in studies on chemical oncogenesis *in vitro*^{5–10}. The cells are highly sensitive to post-confluence inhibition of cell division, show a remarkably low rate of spontaneous transformation and grow with a fibroblast-like morphology with long cytoplasmic processes in sparse cultures. When confluent, they form flat even monolayers and appear epithelioid. Scanning electron microscopy has shown the confluent cells to be extremely thin and polygonal with smooth surfaces resembling epithelial cells¹¹. Here we describe experiments showing that the multinucleated tubular cells which arise in C3H 10T½ C18 cells treated with aza-CR are in fact functional myotubes.

The formation of a large focus of multinucleated cells in a culture of C3H 10T½ C18 cells treated with aza-CR is shown in Fig. 1a. The long tubular characteristics of the cells growing on a monolayer of mononucleated cells should particularly be noted. These tubular cells contained many nuclei with distinct nucleoli (Fig. 1b). The background of C3H 10T½ C18 cells can also be seen.

In some such cells, regular striations were apparent (Fig. 1c), with a repeating unit length of $2.7 \mu\text{m}$. The striations were a feature which appeared shortly after the formation of the multinucleated cells about 9–10 d after treatment with aza-CR. Although the multinucleated cells sometimes persisted for up to 20 d after their appearance, most of them started to atrophy soon after they were

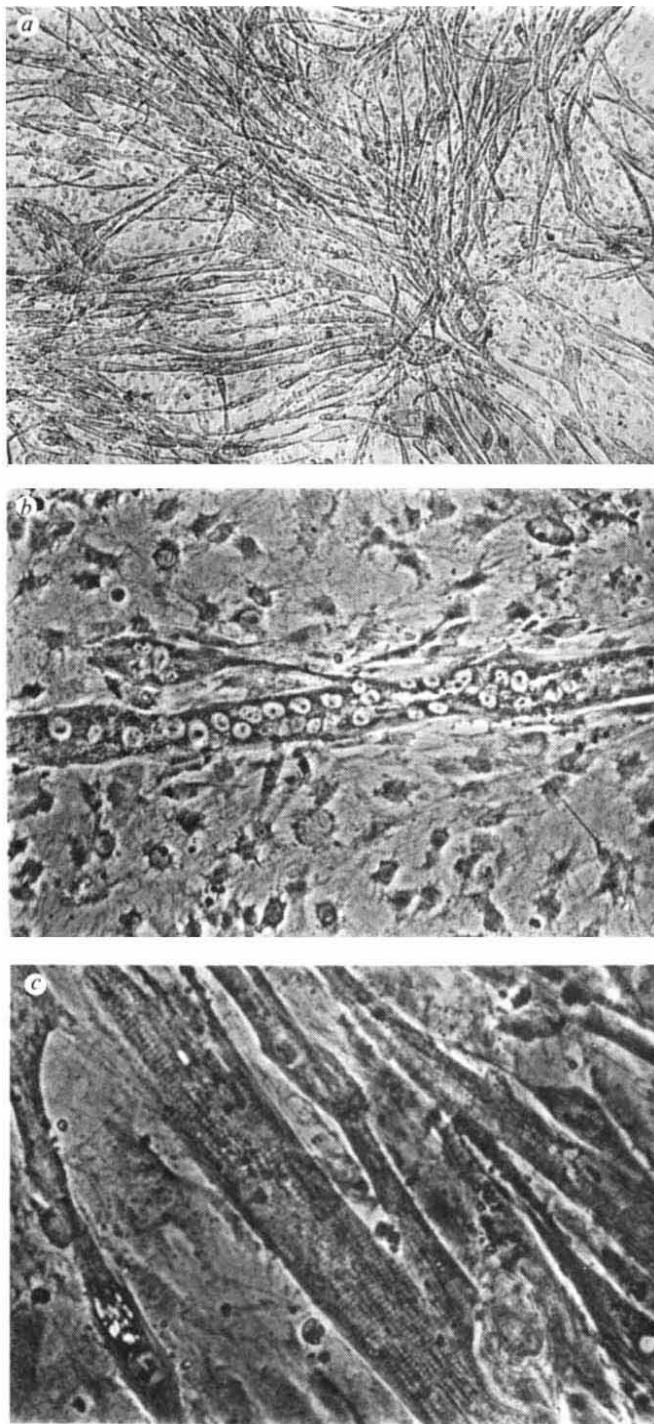


Fig. 1 Myotubes induced in C3H 10T½ C18 cells by aza-CR. *a*, Low magnification ($\times 69$) of a living culture showing a focus of multinucleated cells. *b*, Phase contrast microscopy of a living culture showing many nuclei with distinct nucleoli in a multinucleated cell. The background of evenly-distributed nuclei of the C3H 10T½ C18 monolayer can also be seen ($\times 252$). *c*, Phase contrast microscopy of a living culture showing clearly defined, regular striations ($\times 546$).

formed and not many remained longer than 14 d. Table 1 lists some of the physical characteristics of these cells. They are macroscopic and many can be seen with the naked eye. They are generally long and thin, being on average 20 times longer than they are thick. The tubular cells are found either isolated or in foci (for example, Fig. 1a); however, the majority of multinucleated cells are found in foci. There is a large variation in the number of nuclei per cell, ranging from 2 to approximately 2,000, the average being 8.6 nuclei per cell.

A small percentage of the multinucleated cells have occasionally been seen to contract spontaneously or twitch in culture about 7 d after their appearance. This twitching continued for various lengths of time, between 1 and 7 d depending on the stage of atrophy of the cells. Twitching was generally seen in the middle or late atrophy stages of

We consider these cells which arise in aza-CR treated cultures to be muscle cells for the following reasons: (1) The cells are multinucleated and tubular in morphology. (2) Striations are evenly and regularly spaced and found to be 2.7 μm long. (3) Spontaneous contractions of the cells have been seen in several experiments. (4) The cells

Table 1 Physical characteristics of multinucleated tubular cells arising in aza-CR-treated cultures

Character	Average value	Range	No. of cells examined
Length	0.9 mm	0.16–3.96 mm	23
Width	0.043 mm	0.007–0.37 mm	23
No. of nuclei per cell	8.6	2–2,000	736
Twitching rate	88 contractions per min	55–160 contractions per min	14

The measurements were made on fixed and stained dishes with the exception of the twitching rate which was determined in living cultures.

the life of multinucleated cells, and the twitching rate shown in Table 1 varied from 55 to 160 contractions per min. The twitching was generally localised to small areas of the cells, although waves of contraction have been seen to pass through a length of a focus in one case.

Cells possessing striations have been artificially stimulated to contract with acetylcholine. This was done by placing a drop of 1 mM acetylcholine over the cell in question while observing it with the inverted microscope. This technique was not successful in every instance but some tubular cells contracted strongly and then remained in the contracted state.

The effect of aza-CR was markedly dependent on the concentration of analogue used (Table 2). Little cytotoxicity, as measured by decrease in plating efficiency, was seen with a level of 3.0×10^{-7} M aza-CR and a few multinucleated cells were formed. A concentration of 10^{-6} M aza-CR, however, caused appreciable cell death while there was a very large increase in the number of nuclei appearing in multinucleated cells. A dose of 3.0×10^{-6} M aza-CR was considerably more cytotoxic (80% decrease in plating efficiency) and fewer nuclei appeared in tubular cells. But, when a correction was made for the extent of cell killing, this dose was also effective in inducing the formation of multinucleated cells. Table 2 also shows that the intact triazine ring of aza-CR is necessary for this effect. The ring is rapidly hydrolysed¹² at slightly alkaline pH and we found the half-life of aza-CR to be about 90 min at 50 °C in phosphate-buffered saline at pH 7.4. When the stock solution was kept at this temperature for 20 h it lost its ability to cause cytotoxicity and multinucleation. Finally, it should be noted that there are approximately 600,000 mononucleated cells in the dishes at confluence so that the percentage of nuclei appearing in tubular cells is relatively small even under optimum conditions.

respond positively to acetylcholine which points to the presence of functional acetylcholine receptors. None of these properties has been found in untreated C3H 10T $\frac{1}{2}$ C18 cultures or in cultures treated with many other agents. Furthermore there is no other evidence to suggest that the line is myoblastic in nature^{3,11}. It is unlikely that aza-CR selects for possible myoblast contaminants in the parent line since these effects have been found in a laboratory where myoblasts were not grown⁴ and multinucleated tubular cells are never seen in untreated controls. An additional point is that the multinucleated cells were induced in two separate batches of C3H 10T $\frac{1}{2}$ C18 cells which were obtained from the McArdle laboratory. The effects of aza-CR are reproducible although we have found variations in the absolute numbers of fused nuclei in different experiments.

We therefore conclude that the production of muscle cells was induced by aza-CR. Piled-up foci of other cell types were also formed following treatment with aza-CR. We are currently attempting to characterise them, since it is possible that other types of phenotypic conversion may also occur.

Two possibilities for the origin of the multinucleated cells are (1) endoreduplication and (2) fusion of many mononucleated cells. Preliminary photographic experiments have shown many bipolar mononucleated cells in areas where multinucleated cells subsequently appeared suggesting that fusion was occurring. It seems, therefore, that aza-CR causes regulatory changes in the C3H 10T $\frac{1}{2}$ C18 cells which leads to some of them becoming myoblasts. The precise cell type from which the C3H 10T $\frac{1}{2}$ C18 cell is derived is not known, but this information will be of value in assessing the extent and the nature of the change induced by aza-CR. It seems possible, however, that the compound will be of value in studying differentiation in an analogous way to the use of

Table 2 Formation of multinucleated cells after treatment of C3H 10T $\frac{1}{2}$ C18 cells with aza-CR

Concentration of aza-CR (M)	Plating efficiency (%)	Average no. of nuclei in multinucleated tubular cells* per dish	No. of nuclei in multinucleated cells per 100 cells surviving treatment
0	25	0	0
3.0×10^{-7}	22	133	60
1×10^{-6}	13	4,624	3,557
3.0×10^{-6}	5	1,637	3,274
1×10^{-6} , preheated at 50 °C for 20 h	26	0	0

Stock solutions of aza-CR were made up fresh for each experiment in phosphate-buffered saline pH 7.4, sterilised by filtration and kept at 0 °C until used. C3H 10T $\frac{1}{2}$ C18 cells were used in the 15–20th passage and were grown in Eagle's basal medium supplemented with 10% heat-inactivated foetal calf serum and penicillin and streptomycin. Cells obtained from log-phase mass culture were seeded into 60-mm dishes (1,000 cells per dish) and treated 24 h later with the indicated doses of aza-CR. Exposure was for 24 h, after which the cultures were washed and maintained for 14–16 d. The dishes were then rinsed with water, fixed with methanol and stained with May/Grünwald and then Giemsa stains. The dishes (four per group) were then scored for nuclei in multinucleated cells at 100 $\times$ magnification. Cell killing was determined by the lowering of plating efficiency in similarly treated cultures containing 200 cells and stained with Giemsa stain after 10–12 d. Data given are the average values from two separate experiments.

*A multinucleated tubular cell was defined as an elongated cell having two or more nuclei.

5-bromo-2'-deoxyuridine¹³. In this regard it is of obvious interest that the analogue is incorporated into nucleic acids with a high efficiency¹⁴.

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Voltage-dependent effect of curare at the frog neuromuscular junction

CONDUCTANCE measurements on various cholinergic post-junctional membranes have demonstrated voltage-dependent effects for acetylcholine (ACh) and its agonists¹⁻⁵, for local anaesthetics⁶, and for curare⁷. It is not yet clear which of the steps in drug-receptor interaction are rate-limiting for voltage-dependent effects of ACh. Kordaš⁷ considers this step to be the

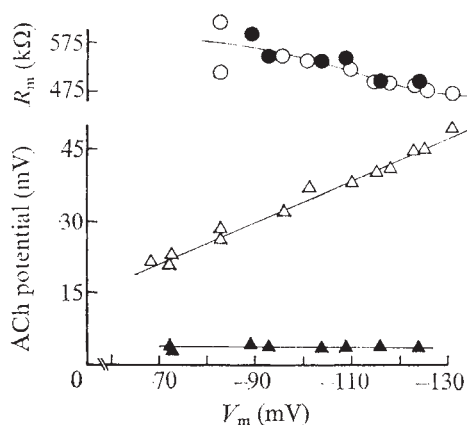


Fig 1 Lower: Peak amplitude of the ACh potential (ordinate) plotted against the membrane potential (V_m , abscissa) for data obtained during the control period (Δ) and then, several minutes later, in the presence of $4.3 \mu\text{M}$ TC-Ringer ($\blacktriangle$). The membrane potential plotted is that value measured just before the beginning of an ACh potential. The amount of ACh released by iontophoresis was constant throughout, the total charge released per iontophoretic pulse being 4.37 nC . The data were obtained while the membrane was hyperpolarised away from and, then, repolarised back to the resting potential. Note that the ACh potential amplitude increased with hyperpolarisation while the preparation was bathed in normal Ringer but that it remained essentially unchanged during hyperpolarisation when curare was present. Upper: The input resistance of the muscle fibre (ordinate) is plotted against membrane potential. Measurements made before ($\circ$) and in the presence of curare ($\bullet$) show that the small degree of anomalous rectification¹³ observed in this fibre was unaffected by curare.

initial binding between ACh and its receptor, whereas Magleby and Stevens⁸ consider the conformational changes between open and closed states of the receptor to be rate-limiting. Distinct from the initial binding reaction and the subsequent conformational change is the possibility of a drug binding to a receptor that is in its open, rather than closed, state, thereby forming a complex consisting of three molecular species. This idea comes from Steinbach's model⁹ for the action of local anaesthetics at the frog endplate. Such multi-molecular complexes have been included in reaction schemes which describe voltage-dependent effects for local anaesthetics at the frog endplate¹⁰ and for curare in *Aplysia* neurones⁵. Marty *et al.*⁵ found that during hyperpolarisation of molluscan neurones, curare became a more effective blocker of the response to slowly applied ACh. Such an effect of curare was not seen when ACh was applied quickly to the receptors, that is when there was insufficient time for ACh to equilibrate with its postsynaptic effect. They suggested that the binding between curare and the cholinergic receptor is

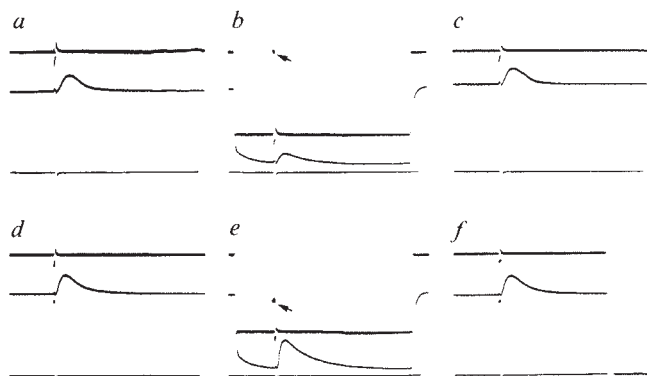


Fig. 2 Reversibility of the increased blocking effect of curare during hyperpolarisation. Six oscillograms (a-f) were obtained sequentially from the same endplate. Three CRO traces are present in each oscillogram. Upper trace, hyperpolarising current (inward current, downward) applied to the fibre from an intracellular microelectrode. Middle trace, intracellular recording of membrane potential. Lower trace, iontophoretic current (outward current, upward) through the ACh pipette. (Arrows point to the tops of the ACh current pulses in b and e.) a-c: ($4.3 \mu\text{M}$ TC-Ringer) A response was first obtained at the resting potential (a). The ACh potential decreased during hyperpolarisation (b) and returned to its control value when ACh was applied again at the resting potential (c). The preparation was then washed with curare-free Ringer, and about 2 min later, the recordings in d-f were obtained. The amount of ACh released by iontophoresis, held constant in a-c, was decreased until the amplitude of the ACh potential in d was about equal to that in a. The ACh potential increased with hyperpolarisation (e). The resting potential was -63.4 mV . Total charge released from ACh pipette: a-c, 18.0 nC ; d-f, 5.74 nC . Horizontal calibration: 400 ms. Vertical calibration: 100 nA (membrane current); 40 mV (V_m); 200 nA (iontophoretic current).

voltage-dependent but that this particular kind of binding can only occur between curare and a receptor which is already in its open state. Here I show that curare has a very similar effect at the frog neuromuscular junction. The efficacy of curare as a postsynaptic blocker at the frog neuromuscular junction increases when the muscle fibre is hyperpolarised. This effect is seen only when ACh is applied slowly, however, and not when it is applied rapidly during neural release of ACh.

Experiments were performed at room temperature ($19-23^\circ\text{C}$) on sartorius muscles from *Rana pipiens*; in some experiments, nerve muscle preparations were used. The composition of the normal Ringer (mM) was: 111 NaCl; 2.5 KCl; 2.0 CaCl_2 ; 0.4 NaH_2PO_4 ; 1.1 Na_2HPO_4 (pH 6.9). Conventional procedures were used for applying ACh by iontophoresis^{11,12}, for recording membrane potential with an intracellular microelectrode (containing 1 M potassium citrate), and for changing membrane potential by passing constant current pulses through a second intracellular microelectrode. The ACh pipettes had tip diameters

of about 1 μm and electrical resistances of 30–60 $\text{M}\Omega$. The oscillograms shown in Fig. 2 are representative of the duration of the constant current pulses used to change the membrane potential and the time course of the responses to iontophoretically applied ACh (ACh potentials). ACh was applied at 15-s intervals. The responses to iontophoretically applied ACh were always much slower (by a factor of about 50–100) than were those due to ACh released from nerve terminals.

Figure 1 shows an experiment in which the amplitude of the ACh potential was measured at various membrane potentials with and without 4.3 μM tubocurarine (TC). In the absence of curare the ACh potential increased together with the driving force as the muscle fibre was hyperpolarised. In the presence of curare, however, the ACh potential, quite unexpectedly, did not increase with hyperpolarisation. This phenomenon is reversible in two respects. First, the increased action of curare was only seen during hyperpolarisation (Fig. 2b); it was not seen when the hyperpolarising pulse was no longer applied (Fig. 2c). Second, when the curare was removed by washing with curare-free Ringer (Fig. 2d–f), the phenomenon was no longer observed (Fig. 2e).

The voltage-dependent effect of curare shown in Figs 1 and 2 is not simply an apparent effect resulting from errors inherent in the measurement of postsynaptic potentials due to the non-linear electrical characteristics of the muscle fibre¹³ and/or to the fact that the instantaneous driving force actually decreases during a postsynaptic potential. Previous work¹⁴ and data from this study (Figs 1 and 2b, e) show that curare does not affect the electrical properties of the nonsynaptic membrane. In addition, when the iontophoretic current was adjusted so that ACh potentials of almost equal amplitudes were obtained at the resting potential both in the presence and absence of curare (Fig. 2a, d), the ACh potential decreased during hyperpolarisation only when curare was present (Fig. 2b).

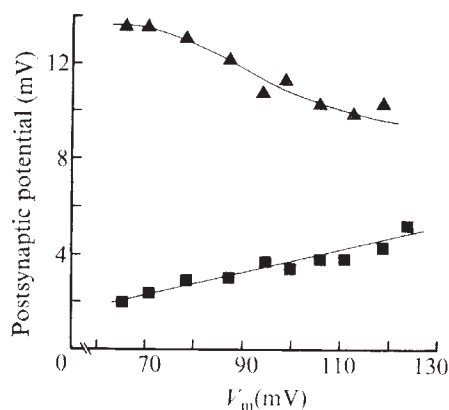
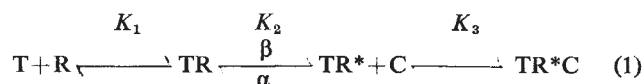


Fig. 3 Comparison of the effects of hyperpolarisation on the ACh potential and e.p.p. in the presence of curare. The amplitudes of the postsynaptic potentials due to the iontophoresis of ACh (ACh potentials, ▲) and to the synchronous release of ACh quanta from nerve terminals (e.p.p.s, ■) are plotted on the ordinate against membrane potential (abscissa). All data are from the same endplate. 3.6 μM TC was present. The ACh potentials and the e.p.p.s were recorded on alternate sweeps as the muscle fibre was hyperpolarised. Either the nerve was stimulated or ACh was applied by iontophoresis approximately 400 ms after the start of a hyperpolarising pulse. The e.p.p. increased with hyperpolarisation, as expected, while the ACh potential decreased. The increased action of curare with hyperpolarisation was not seen with neurally released ACh; this action was only observed when ACh was applied more slowly to the receptors by iontophoresis. In this experiment, the input resistance decreased from 800 $\text{k}\Omega$ during minimal hyperpolarisation to 400 $\text{k}\Omega$ during maximal hyperpolarisation. Therefore, there is a possibility that, due to the complex electrical properties of the muscle fibre^{9,17}, anomalous rectification influenced only the slow ACh potential and not the fast e.p.p.

The simplest model to account for this voltage-dependent effect of curare would involve an increased binding between curare and the closed cholinergic receptor during hyperpolarisation. But, if that were true, then the response to ACh released from nerve terminals should also reflect this increased binding of curare. The work of Takeuchi and Takeuchi¹⁵, and data in Fig. 3 show that such a voltage-dependent effect of curare is absent in experiments dealing with the neural release of ACh. In the experiment shown in Fig. 3, simultaneous recordings were made of the endplate potential (e.p.p.; neural release of ACh) and the ACh potential (iontophoresis of ACh). During hyperpolarisation the e.p.p. increased in a manner similar to that seen for neurally evoked endplate currents¹⁵, while the ACh potential decreased.

Thus, we can reject the possibility that curare binds more effectively to the closed receptors during hyperpolarisation, and conclude that there must be two mechanisms for the action of curare. One mechanism is dependent on membrane potential and the other is not. The first mechanism is the classical one¹⁶ in which curare binds to the closed cholinergic receptor and competes with ACh for binding sites; the classical action of curare is voltage-independent. The second mechanism, described in this study (see also ref. 5), might involve curare binding to the ACh receptor after it has already undergone a conformational change. Since an ACh receptor undergoes a conformational change during its interaction with ACh, I suggest that it is the activated ACh receptor complex⁹ (open state of the receptor) to which curare can bind with increasing effectiveness during hyperpolarisation. The proposed reaction scheme, which is based on that given by Ruff¹⁰ for the effect of a local anaesthetic on ACh-receptor-interaction, is



where K_1 represents the initial binding between agonist and receptor, K_2 represents the conformational change to an open receptor (TR^*), and K_3 represents the voltage-dependent binding reaction between the open receptor and curare to form TR^*C . It is assumed that the conductance of TR^*C would be zero.

Neural release of ACh is so fast that ACh is not in an equilibrium with its postsynaptic effect, whereas such an equilibrium can be attained when ACh is slowly applied by iontophoresis^{1,18,19}. It is of interest to point out a similarity between the voltage-dependent effect for ACh (that in which the ACh-induced conductance increases with hyperpolarisation^{1–5,20}) and that for curare. Both effects are observed only under equilibrium conditions, that is, both are absent when ACh is released quickly from nerve terminals (Fig. 3; refs 1 and 15). One may then ask if similar mechanisms are involved. Thus, can an activated agonist–receptor complex bind slowly not only to local anaesthetics and to antagonists, but also to agonists?

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Immune lesions of noradrenergic neurones in rat central nervous system produced by antibodies to dopamine- β -hydroxylase

THE development of antibodies against several neurotransmitter synthesising enzymes has facilitated immunohistochemical identification of specific neurones in the nervous system^{1,2}. The availability of these antibodies has also raised the possibility of producing immune lesions of the neurones in situations where the antibody has access to its antigen *in vivo*. In the case of synaptic vesicle antigens, the vesicle membrane becomes incorporated into the nerve terminal membrane during release of transmitter by exocytosis^{3–5}. Dopamine- β -hydroxylase (DBH), the enzyme that synthesises noradrenaline, is located in the membrane of noradrenergic synaptic vesicles and would become exposed to antibodies in the extracellular space during nerve activity⁶. Binding of injected DBH antibodies has been demonstrated both histochemically and biochemically in sympathetic nerves^{7–9}. Antibodies to DBH cause degeneration of noradrenergic nerve terminals in several guinea pig tissues when injected intravenously^{10,11}; this form of immunosympathectomy seems to be a complement-mediated reaction¹². We report here that injection of anti-DBH serum and complement into the cerebrospinal fluid (CSF) of rats produces degeneration of noradrenergic nerve fibres in the central nervous system.

Female Porton rats (200–250 g) were anaesthetised with intraperitoneal sodium pentobarbital (42 mg per kg body weight) and placed in a Kopf stereotaxic apparatus. A 21 or 23 gauge steel needle connected to a Harvard infusion pump was inserted stereotactically in either a lateral or the third ventricle. Rabbit anti-bovine DBH serum¹³ (50–250 μ l) mixed with complement (50–250 μ l of guinea pig serum) was infused over 1–2 h. Outflow of CSF and antiserum occurred from the subarachnoid space around the infusion site or from a cannula inserted in the cisterna magna. Nine rats received anti-DBH serum and complement and four control rats received equivalent volumes of non-immune rabbit serum and complement.

At times varying from 2 to 7 d after antibody infusion, rats were anaesthetised, a cannula inserted retrogradely into the abdominal aorta and the right atrium was cut. Perfusion was started with normal saline and continued for 1 h with 4% formaldehyde and 0.5% glutaraldehyde (electron microscopy grade) in 0.1 M sodium phosphate buffer, pH 7.0. This aldehyde mixture has recently been shown to fix the nervous system and simultaneously convert catecholamines to fluorescent derivatives¹⁴. The brain and upper spinal cord were removed, cut into 5 mm transverse slices, and 30- μ m sections cut on a Vibratome at 4 °C. The freshly cut sections were photographed at low magnification using transmitted white light and dried over phosphorus pentoxide for 1 h. The sections were then mounted in liquid paraffin or Fluormount and examined with a Leitz Ortholux II microscope, fitted with a high pressure mercury vapour lamp. Both control and experimental sections were studied immediately after preparation of the sections before fading of the finest terminals. The distribution of fluorescent, catecholamine-containing structures was mapped on prints of the original white light photographs¹⁴.

Many thick and distorted fluorescent axons were visible throughout the brains of all animals that received immune serum (Fig. 1a). They were most numerous in the medial septal nuclei, diagonal band of Broca, piriform cortex,

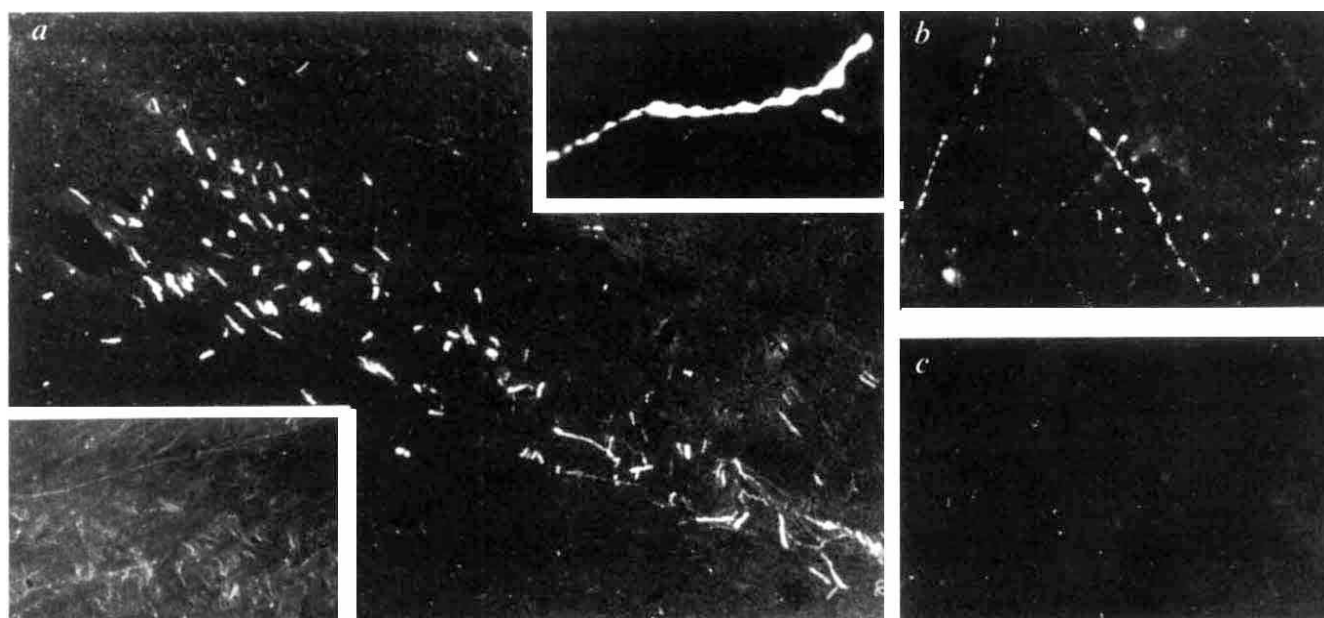


Fig. 1 *a*, Fluorescence photomicrograph of a transverse section through the zona incerta of a rat brain 3 d after anti-DBH serum and complement were infused into the third ventricle. Many obliquely sectioned noradrenergic fibres are thick and distorted. ($\times 165$.) Insert top right: a single degenerating axon in the same area. ($\times 400$.) Insert lower left: zone incerta of normal rat brain showing oblique sections of bundles of ascending noradrenergic axons. ($\times 165$.) *b*, Fluorescence photomicrograph of a transverse section through the hippocampus of a rat brain 3 d after non-immune serum and complement were infused into the lateral ventricle. Many normal varicose axon terminals are present. ($\times 330$.) *c*, A corresponding hippocampal area in a rat 3 d after anti-DBH serum and complement were infused into the lateral ventricle. The noradrenergic terminals are no longer apparent. ($\times 330$.)

rostral corpus callosum, zona incerta, posterior commissure, dorsal tegmental bundle and occasionally in fibre tracts in the lateral funiculi of the thoracic spinal cord. In contrast to the fine smooth appearance of normal catecholamine-containing axons, the degenerating fibres were very brightly fluorescent and irregularly swollen. In longitudinal sections the axons often appeared as a series of bulges connected by fine strands.

In addition to the swollen axon bundles, there was a patchy loss of varicose axon terminals accompanied by occasional swollen varicosities in the neocortex, hippocampus and cerebellar cortex (Fig. 1c). The catecholamine-containing cell bodies in the brain stem seemed normal. In the control rats, there was no detectable loss of varicose axon terminals (Fig. 1b) and the only swollen fibres seen were confined to the area of local necrosis along the pathway of the cannula.

The distribution of the swollen fluorescent fibres occurs within the general distribution of known noradrenergic fibre tracts¹⁵⁻¹⁷, and their appearance resembles that of degenerating noradrenergic axons in the peripheral nervous system induced by antibodies to DBH (ref. 10). The immune lesions are probably produced when antibodies bind to DBH exposed in the axon terminal membrane during exocytotic release of transmitter with subsequent lysis of the terminal membrane by complement fixed by the immune reaction¹². This destruction of axon terminals leads to an accumulation in preterminal axons of noradrenaline-synthesising vesicles transported from the neurone cell body⁶.

The lesions in the central nervous system are likely to be confined to noradrenergic and adrenergic neurones in which DBH is localised. The specificity of the antiserum used has been well defined by both immunochemical⁶ and immunohistochemical procedures¹⁰ and, in the peripheral nervous system, the degeneration produced by intravenously administered anti-DBH was confined to noradrenergic nerves¹². In principle, the use of an antiserum directed against a specific constituent of synaptic vesicle membranes could be extended to produce immune lesions of neurones using transmitters other than noradrenaline.

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Possible role of pH gradient and membrane ATPase in the loading of sucrose into the sieve tubes

For plants to grow, sugars and other assimilates must constantly be transported from the leaves to the growing or storage regions. This essential transport function takes place in the highly specialised phloem tissue which consists of a network of interconnecting sieve tubes. In spite of its obvious agronomic importance, little is known about the first step of sugar entry (loading) into the sieve tubes. Here I propose a model which formulates several of the established, but difficult to reconcile, characteristics of the sieve tubes into a unifying model for sugar uptake. These characteristics include the alkaline sap¹ (pH 8-8.5) with a high potassium ion and ATP² concentration, the presence

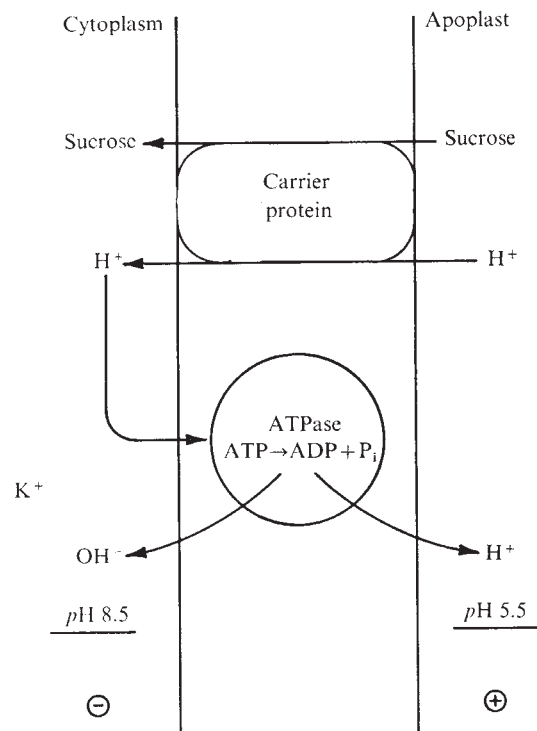


Fig. 1 Model of transport into sieve tubes

of electrical gradients³ and the considerable ATPase⁴ activity of the sieve tube plasmalemma. According to this model, sucrose leaves the site of synthesis in the mesophyll cells and enters the free space or apoplast which has a pH of 5 to 6. The sucrose is then accumulated into the alkaline sieve tubes by an active process involving membrane sulphhydryl groups⁵. In this model the proton gradient of nearly 3 pH units across the sieve tube plasmalemma provides the driving force for sucrose uptake (Fig. 1). Based on non-electrolyte transport studies in bacteria⁶, algae⁷ and fungi⁸ it is proposed that protons are cotransported with sucrose into the sieve tubes.

The proton gradient across the membrane is maintained for continuous sucrose influx by an ATP-driven proton extrusion system wherein ATP is hydrolysed by the plasmalemma ATPase in such a way that an asymmetrical distribution of protons and hydroxyl ions is established across the sieve tube plasmalemma thereby maintaining the internal alkalinity. Studies on the cotransport of hexose and protons in the algae⁷ and fungi⁸ indicate that both a proton gradient and its resulting electrical potential are

involved in uptake. In this regard, it should be noted that potassium ions have been implicated in electrical gradients in the phloem³. Thus, it would be most valuable to establish the magnitude of any electrical potential difference across the sieve tube membrane during sucrose uptake.

Data on phloem loading of sucrose support the model. Using non-permeant buffers to change the pH of the apoplast, sucrose uptake into the sieve tube and translocation from the source leaf of an intact plant were inhibited when the magnitude of the pH difference between the apoplast and symplast was reduced⁹. The pH optimum for phloem loading is 5, and loading is markedly inhibited at pH 8. In addition, the affinity of the carrier for sucrose is decreased at pH 8. Thus, proton and sucrose molecules can bind to a membrane carrier which displays a higher affinity in the protonated form (Fig. 1). The charged complex (sucrose-H⁺-carrier) can be driven across the membrane by the electrical potential difference generated by the proton extrusion mechanism.

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Echovirus 12-induced host cell shutoff is prevented by rhodanine

INFECTION of animal cells with picornaviruses results in inhibition of host cell RNA and protein synthesis shortly after infection, as was shown with members of the cardiovirus group¹⁻³, with poliovirus⁴⁻⁶, and with foot-and-mouth disease virus⁷. The molecular mechanism of this so-called shutoff is not well understood; however, it seems to require protein synthesis early in infection^{2,8,9}, but no viral RNA replication⁹⁻¹¹. We report here that 2-thio-4-oxothiazolidine (rhodanine), at a concentration which inhibits selectively the uncoating of echovirus 12 in GMK cells also inhibits the virus-induced shutoff of GMK cell protein synthesis.

The infection of GMK cells, a permanent cell line derived from African green monkey kidney cells¹², with echovirus 12 (prototype Travis) is followed by inhibition of the GMK cell protein synthesis to the extent of 80% by 3 h post infection (p.i.). A clear peak of viral protein synthesis is visible 4-5 h p.i. RNA synthesis declines more gradually, showing a specific shoulder 4-5 h p.i. due to viral RNA synthesis. At 3 h p.i. viral RNA and protein synthesis is not yet detectable in the conditions of our experiments and formation of new particles has not yet started¹².

Rhodanine acts by blocking uncoating of the virus without inhibiting virus adsorption or penetration¹². Nor does it affect the replication of purified echovirus 12 RNA in GMK cells. Furthermore, infective virus could be recovered quantitatively from infected, rhodanine-treated cells and rhodanine was shown to protect the echo 12 virion from alkaline degradation *in vitro*. Rhodanine apparently acts by stabilising the virion structure. We also expected to find that rhodanine would inhibit the virus-induced shutoff of host cell protein synthesis. Indeed, our experiment showed that no host shutoff takes place in echovirus 12-infected cells in the presence of rhodanine, whereas in the untreated

control host cell protein synthesis is repressed to less than 20% of the mock-infected control by 3 h p.i. (Fig. 1).

In contrast the shutoff induced by poliovirus 2 (strain P 712-ch-2ab) was not affected by rhodanine (Fig. 1). That is consistent with the fact that the multiplication of poliovirus 2, unlike that of echovirus 12, is unaffected by rhodanine^{12,13}.

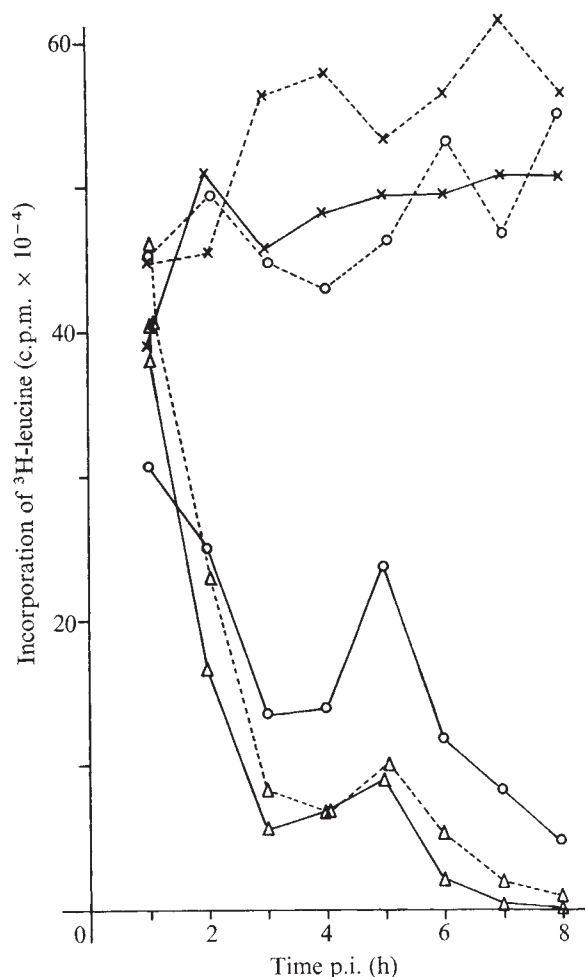


Fig. 1 Confluent monolayers of GMK cells were infected with echovirus 12 (20-50 plaque-forming-units (PFU per cell) or poliovirus 2 (50-100 PFU per cell) in the absence or presence of 100 $\mu\text{g ml}^{-1}$ rhodanine. Rate of protein synthesis was measured as follows: L-(4, 5-³H)-leucine was added to an aliquot of the cells at various times p.i. The cells were collected 30 min later, and the radioactivity incorporated into trichloroacetic acid precipitable material was determined. Broken lines denote presence of rhodanine, solid lines denote absence of rhodanine. $\times$, Mock infection; $\circ$, echovirus infection; Δ , poliovirus infection.

The shutoff effect by echovirus 12 is, within experimental limits, as dependent on the multiplicity of infection as it is with poliovirus 2 (Fig. 1). We do not know whether the effects of rhodanine would persist at still higher multiplicities. This experiment might be complicated by emergence of rhodanine-resistant or -dependent mutants¹².

This result demonstrates that the specific inhibitor of uncoating of echovirus 12, rhodanine, also prevents the virus-induced shutoff of host protein synthesis. Obviously, adsorption and penetration are not sufficient to induce the effect; uncoating is a necessary prerequisite for this shutoff to occur. The virion per se—for example, by depressing some cellular function(s)—is not able to induce host cell shutoff.

Rhodanine thus seems to fulfil some of the ideals

clinicians ask for in viral chemotherapies—it affects an early stage of virus-cell interaction leaving intact vital functions of the cell.

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Guanosine tetra- and pentaphosphate synthesis by bacterial stringent factor and eukaryotic ribosomes

AMINO acid starvation of *rel⁺* bacteria¹ results in a rapid pleiotropic response involving the cessation of stable RNA synthesis, a decrease in the rate of nucleoside transport, an altered pattern of mRNA synthesis, inhibition of phospholipid synthesis and increased protein turnover. This stringent response² is rapidly reversed when the amino acid starvation is relieved and is apparently mediated by two unusual nucleotides, guanosine 5'-diphosphate, 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate, 3'-diphosphate (pppGpp)³. These nucleotides are synthesised on the ribosome in the presence of uncharged tRNA, ATP, GTP and an enzyme known as stringent factor³. Mutants which fail to exhibit the stringent response to amino acid starvation with the synthesis of ppGpp pppGpp are known as relaxed mutants⁴ and in *Escherichia coli* are mapped at three loci; *relA* (ref. 5), *relB* (ref. 6), and *rplK* (*relC*) (refs 7 and 8). The synthesis of ppGpp and pppGpp has also been observed in various prokaryotes including blue-green algae⁹. Here we demonstrate the ability of bacterial stringent factor to be stimulated by eukaryotic ribosomal preparations to synthesise both ppGpp and pppGpp.

In eukaryotic cells, viral transformation results in the loss of regulation of many macromolecular processes involved in the response of resting cells to serum deprivation¹⁰. This loss in regulation is reminiscent of that observed in relaxed bacteria. Hershko *et al.*¹⁰ suggested that normal fibroblasts and virally transformed fibroblasts were analogous to stringent and relaxed bacteria respectively. Attempts to find ppGpp and pppGpp in eukaryotic cells have been generally unsuccessful^{11–13}, however, although in the last 2 yr a number of reports describing the accumulation of these compounds have been published, both *in vivo*^{14,15} and in ribosome dependent reactions *in vitro*^{16,17}.

Our inability to duplicate the synthesis of ppGpp in CHO cells as reported by Rhaese¹³ and failure to detect ppGpp and pppGpp in a temperature sensitive (*ts*) leucyl-tRNA synthetase mutant of CHO cells at either permissive or non-permissive temperatures¹³, led us to examine the *in vitro* ribosome-dependent reaction reported by Irr *et al.*¹⁷. In this reaction there might be less chance of a rapid degradation of any ppGpp or pppGpp synthesised. Preliminary attempts to duplicate the work of Irr *et al.*¹⁷ using 11- and 14-d-old mouse embryo ribosomes were unsuccessful (Table 1), using either the ribosome assay described by Irr *et al.*¹⁷ or the assay described by Haseltine and Block¹⁸. Addition of purified stringent factor¹⁹ from *E. coli*, how-

ever, resulted in the synthesis of ppGpp and pppGpp (identified by the co-migration with authentic bacterial ppGpp and pppGpp) converting up to 80% of the GTP over a 2-h incubation period into these guanosine nucleotides (Fig. 1, Table 1). The synthetic reaction observed was slightly different from that seen for *E. coli* ribosomes in that the preparations of eukaryotic ribosomes were rapidly able to convert GTP to GDP, which is used in the synthesis of ppGpp (Fig. 1). Synthesis of ppGpp and pppGpp was dependent on ribosomes, GTP, ATP and stringent factor, omission of any one produces less than 0.1% conversion of the added GTP (Fig. 1). Ribosome preparations were not purposely depleted of bound tRNA and mRNA¹⁸, because we desired to keep the mammalian system as intact as

Table 1 Ability of eukaryote ribosomes to synthesise guanosine penta- and tetraphosphate in an *in vitro* ribosome assay

Ribosome source	µg RNA per assay	Reaction time (h)	% of GTP converted to ppGpp and pppGpp	
			Stringent factor 40 µg ml ⁻¹	0 µg ml ⁻¹
<i>E. coli</i> salt washed	9.3	2	92.7	<0.1
11-d mouse embryo	1	11.0	2	1.1
	2	44.0	2	44.0
	3	14.0	3	61.0
14-d mouse embryo	1	52.0	2	81.3
	2	52.0	2	18.0

Assays were performed as described in Fig. 1. Ribosomes were prepared by the method of Irr *et al.*¹⁷, except the ethanol precipitation step was omitted in all preparations other than the first 11-d mouse embryo experiment. Ribosomal integrity was confirmed by linear (10–40% w/w) sucrose gradient sedimentation analysis²⁰.

possible. Therefore, dependency on these factors was not tested although they were routinely added to the assay.

Lack of a putative stringent factor activity in eukaryotic ribosomes could be accounted for by its lability during the purification procedure for ribosomes described by Irr *et al.*¹⁷. Therefore, ribosomes with various degrees of purity and prepared in other manners were also tested in the *in vitro* assay. We also tested ribosomes prepared from several other eukaryotes. In no case was a stringent factor independent reaction observed, although ribosomes from all sources tested could be stimulated to synthesise ppGpp and pppGpp upon the addition of stringent factor from *E. coli* (Tables 1 and 2). This test also included ribosomes from a *ts* mutant of leucyl-tRNA synthetase in CHO cells²⁰ (Table 1) supporting the *in vivo* data indicating the lack of synthesis of ppGpp and pppGpp in this cell line¹³. The extent of reaction was rather variable (Tables 1 and 2) because ribosomes were not prepared to maintain maximal function but rather by a variety of methodologies in the hope of achieving the independent synthesis of ppGpp and pppGpp by eukaryotes. Therefore quantitative comparisons between groups cannot be made.

Mitochondria from rat liver have been reported to synthesise ppGpp and pppGpp upon the addition of bacterial stringent factor¹⁶. In the present experiments, however, we believe that mitochondrial ribosomes could not be responsible for more than a small percentage of the activity for the following reasons; (1) ribosomes were routinely prepared under conditions where all mitochondria should have been removed, (2) ribosomal preparations showed no 70S peak (<1%) on sucrose gradients even when large quantities of ribosomes were layered on to gradients for preparative purposes.

It is quite possible that the reaction we observed is relatively nonspecific. Crude preparations of *E. coli* stringent factor in the absence of ribosomes can be stimulated to synthesise ppGpp and pppGpp by 20% methanol²¹, although not under the assay conditions employed here (J. Parker, unpublished). Christiansen and Nierhaus²² have shown stimulation of ppGpp and pppGpp synthesis by stringent factor with a variety of *E. coli* ribosomal proteins.

It may be pertinent to point out that immunological cross-reactivity occurs between chicken liver and *E. coli* ribosomal proteins L7 and L12 (ref. 23).

Richter¹² reported that yeast, reticulocyte and calf brain ribosomes were not stimulated by *E. coli* stringent factor. Beres and Lucas-Lenard²⁴ could not stimulate Ehrlich ascites cell ribosomes, but could stimulate wheat germ ribosome with *E. coli* stringent factor. But, as we show here, ribosomes from various sources were stimulated by *E. coli* stringent factor although some of the yeast preparations were inactive. Although the reasons for these discrepancies are not clear, the stringent factor routinely used in the present report was greater than 50% pure whereas in both other reports relatively crude preparations were used. In our hands even a crude preparation of stringent factor could stimulate eukaryotic ribosomes to

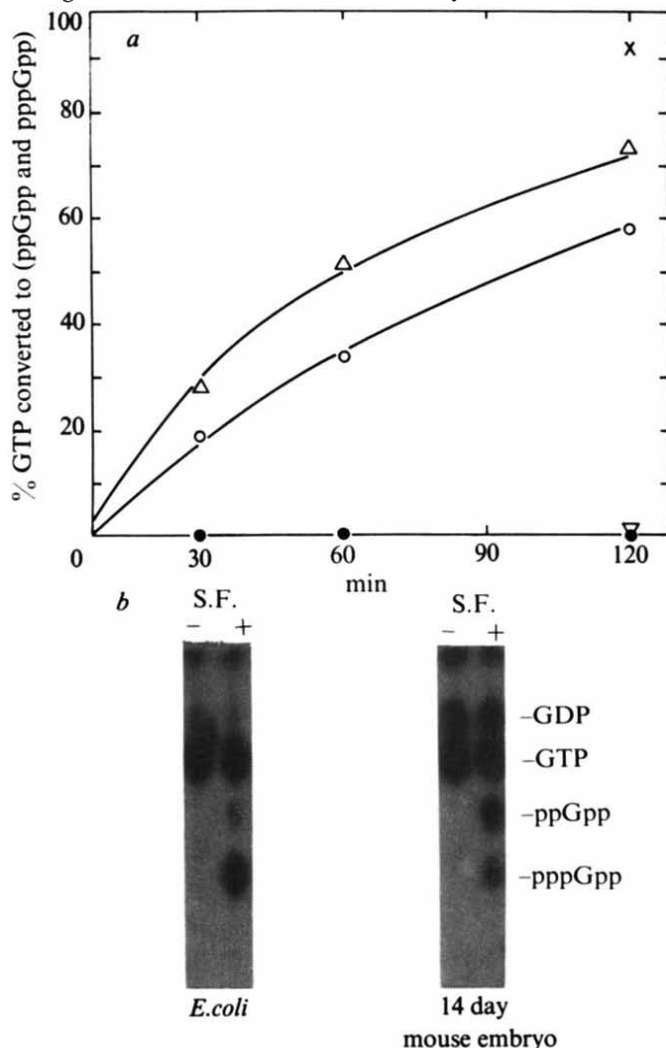


Fig. 1 *In vitro* synthesis of ppGpp and pppGpp by 14-d-old mouse embryo ribosomes and *E. coli* ribosomes in the presence of bacterial stringent factor. *a*, Time course of synthesis of ppGpp and pppGpp. Assays were performed as those of Haseltine and Block¹⁸. 14-d mouse embryo ribosomes were present at a final concentration of 10.4 μg per 50 μl (○), 24 μg per 50 μl (Δ) and *E. coli* ribosomes at a final concentration of 9.3 μg per 50 μl (x). Two μg per 50 μl of at least 50% pure *E. coli* stringent factor (Pedersen and Parker, unpublished) was added where indicated and the reaction mixtures incubated at 37 °C for varying times. The assay lacking ribosomes (●) or lacking *E. coli* stringent factor (▽) gave no detectable synthesis of ppGpp or pppGpp (<0.1% of α -³²P-GTP (Amersham Radiochemicals, specific activity > 1 $\mu\text{Ci } \mu\text{mol}^{-1}$) present at 27.5 nmol per 50 μl assay). *b*, A characteristic polyethylene-imine cellulose chromatogram¹⁸ (Brinkman Instruments) of the reaction products of the assay described in (*a*). *E. coli* ribosomes were present at a final concentration of 9.3 μg per 50 μl and 14-d mouse embryo ribosomes at a final concentration of 26.0 μg per 50 μl . Reaction mixtures were incubated at 37 °C for 2 h.

Table 2 Ability of cytoplasmic ribosomes from various sources to synthesise guanosine penta- and tetraphosphate in an *in vitro* ribosome assay

Ribosomes	Synthesis of ppGpp and pppGpp	
	Exogenous stringent factor 40 $\mu\text{g ml}^{-1}$	0 $\mu\text{g ml}^{-1}$
<i>E. coli</i> crude	+	+
<i>E. coli</i> salt washed	+	—
Hamster embryo fibroblasts*	+	—
Polyoma transformed hamster embryo fibroblasts*	+	—
CHO wild type cells†	+	—
<i>ts</i> leucyl-tRNA synthetase mutation of CHO cells permissive temperature 34 °C	+	—
non-permissive temperature 38.5 °C‡	+	—
Tetrahymena	+	—
Yeast	+	—
Rat liver§	+	—

Assays were performed as described in Fig. 1. Stringent factor was present at 40 $\mu\text{g ml}^{-1}$, except with exogenous *E. coli* ribosomes, where no stringent factor was added. Omission of stringent factor gave no detectable spots of ppGpp or pppGpp or autoradiograms and no counts above background (—). The sensitivity of the assay would have detected 27.5 pmol of ppGpp or pppGpp. Positive results (+) ranged from three times background (rat liver) to three hundred and fifty times background (yeast). Ribosomes from tetrahymena, yeast and rat liver were kindly provided by Mr Peter Ganz, Dr W. Galen McKeever and Dr Doris M. Nicholls, York University, Toronto, respectively.

*Ribosomes were prepared by the method of Irr *et al.*¹⁷

†Cells homogenised as described by Stanners and Thompson¹³ and nuclei removed by centrifugation twice at 650g for 3.5 min and mitochondria once at 7,000g for 10 min. A microsomal fraction was obtained by centrifugation for 1.5 h at 105,000g. Pellets were resuspended in buffer C (0.05 M Tris-acetate pH 8.0 at 10 °C, 0.015 M magnesium acetate, 0.06 M potassium acetate, 0.27 M ammonium acetate, 0.001 M EDTA, 0.0001 M dithiothreitol) and frozen at –70 °C until used.

‡Cell supernatants prepared as described by Stanners and Becker²⁹ were layered over 34 ml linear sucrose gradients (15–30% w/w) in 0.01 M Tris-HCl pH 7.4 at 20 °C, 0.1 M NaCl, 0.02 M MgCl₂ and centrifuged for 2.5 h at 27,000 r.p.m. in a SW27 rotor at 4 °C. The monosome and polysome fractions were collected in a LKB fraction collector after monitoring absorbance at 260 nm. Purified ribosomes were concentrated by centrifugation for 14 h in a SW41 rotor at 41,000 r.p.m., resuspended in buffer C and frozen at –70 °C until used.

§The rat liver ribosome preparation converted 91% of the added α -³²P-GTP c.p.m. to inorganic ³²P-phosphate during the course of the reaction.

synthesise ppGpp and pppGpp (unpublished observations).

In conclusion, we find eukaryotic ribosomes are unable to synthesise guanosine penta- and tetraphosphate *in vitro* except in the presence of bacterial stringent factor. Although several methods of ribosome preparation were used to attempt to maintain the activity of a postulated eukaryotic stringent factor no stringent factor activity was found. We cannot, however, rule out the possibility that such a factor exists but is extremely labile. The majority of the *in vivo* experiments^{11–13, 25–28} and our unpublished results argue against this possibility. In light of the present experiments, previous positive results in *in vivo* eukaryotic experiments may well have resulted from prokaryotic contamination of animal cells.

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Quenching of chlorophyll fluorescence by β -carotene

CAROTENOID pigments are closely associated with chlorophyll molecules in the chloroplast structure^{1,2}. Some carotenoids, for example fucoxanthol³, can act as efficient sensitizers for the formation of the excited singlet state of chlorophyll although others, such as β -carotene, are very inefficient^{2,4,5}. Other functions of carotenoids include acting as a deactivator of triplet chlorophyll (and hence protect the chlorophyll from photodegradation and also intercept the production of singlet oxygen) and as a deactivator of singlet oxygen⁶. Here we report *in vitro* experiments which unequivocally demonstrate that β -carotene quenches the excited singlet state of chlorophyll.

A previous report of the quenching of chlorophyll fluorescence by β -carotene⁷ has been shown to be in error^{8,9}. From a simple consideration of the Stern–Volmer quenching kinetics it can be shown that to measurably quench the chlorophyll *a* excited singlet state of lifetime in benzene 5.73 ns, a quencher concentration of $>10^{-3}$ M is required and thus a report¹⁰ of observed quenching of chlorophyll fluorescence by β -carotene at a concentration of 1×10^{-5} M also seems to be in error.

Steady state, quantitative measurements of the quenching of chlorophyll fluorescence by β -carotene are complicated by the fact that the absorption spectra of the two compounds overlap at wavelengths appropriate for excitation of the chlorophyll and also there is overlap of the β -carotene absorption band with the chlorophyll fluorescence. It is therefore necessary to make extensive corrections to the observed fluorescence intensities. The reported results are based on measurements made with 1 cm² cells and cylindrical cells with an internal diameter of 0.2 cm.

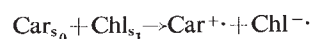
Chlorophyll *a* was used as soon as possible after extraction from *Poa annua* and its purity checked by comparing the relative intensities of its absorption bands at 410, 430, and 662 nm with those in the literature¹¹. For some measurements, chlorophyll *a* from Sigma Chemicals was used: spectral properties of the extracted and commercial samples were identical. Synthetic all *trans* β -carotene (Sigma) was used and its purity checked by measuring the extinction coefficient of its absorption band at 450 nm. The shape of the absorption curve matched, in details, with the literature spectrum for the compound¹¹. The absorption

spectrum of the β -carotene used in the experiments was independent of the concentration used. For all the concentrations used there was no apparent deviation from Beer's law. The linearity of the Stern–Volmer is a further indication that β -carotene does not aggregate at the concentrations employed. Spectrograde benzene (BDH and Merck Uvasol) was used as solvent. For measurements in the square cells, the chlorophyll solution had an optical density of 0.39 at the excitation wavelength (662 nm). The fluorescence intensity was measured at 670 nm. In all cases the solutions were air-saturated. The results of using the square and cylindrical cells are shown in Fig. 1. The agreement between the two sets of results is not very good but the values show that the quenching process has a rate constant of $\sim 5 \times 10^9$ M⁻¹ s⁻¹ which is close to the diffusion controlled limit of 1×10^{10} M⁻¹ s⁻¹ (ref. 13).

To check the steady state quenching data, measurements were made of the quenching effect of β -carotene on the lifetime of the excited singlet state of chlorophyll *a*. Fluorescence lifetimes were measured using time correlated single photon counting¹⁴. The solutions were excited at 550 nm and the emission at 670 nm monitored with a Phillips 56 TUVF photomultiplier tube. The fluorescence was observed at the back of a 1 mm path length cell. In all cases the fluorescence decays were exponential. The results are shown in Fig. 1. The rate constant for quenching, 4.5×10^9 M⁻¹ agrees well with the steady state measurements.

A surprising observation was that the admixing of β -carotene and chlorophyll *a* in aerated solution gave rise to chemiluminescence. The emitting species (as shown by its spectrum) is the excited singlet state of chlorophyll. This luminescence may arise as a result of the thermal dissociation of a β -carotene oxidation product (perhaps a 1,2-dioxetane) to give products in their excited singlet state. Förster-type energy transfer from these excited molecules to β -carotene should occur and ultimately some of the energy be trapped by the chlorophyll lower excited singlet state from which fluorescence occurs.

Since the energy of the excited singlet state of chlorophyll lies below that of β -carotene, the quenching of chlorophyll fluorescence cannot be attributed to an energy transfer process. From the fact that polyenes such as β -carotene have low ionisation potentials and are known to act as electron donors towards excited singlet states¹⁵, we propose that the quenching occurs via an electron transfer process,



The initially produced intermediate may be a non-emissive exciplex. If the quenching occurs in a polar environment radical ions should be produced. Whether or not these can be used in the photosynthetic process is dependent on a number of factors. Of particular importance is the likelihood of back electron transfer before the ions have diffused out of the solvent cage in which they were generated. This type of reaction readily occurs when the radical ions are generated from excited singlet states^{16,17}. We have proposed a similar mechanism to account for the quenching of methylene blue fluorescence by β -carotene¹⁸. Since there is only a little evidence¹⁹ for chlorophyll *a* acting as an electron acceptor in its singlet state we studied the quenching of its fluorescence by other donors, *N,N*-dimethylaniline, indole and skatole. The quenching rate constants for the compounds in methanol solution were found to be 3.4, 0.3 and 0.3×10^8 M⁻¹ s⁻¹ respectively and for indole in benzene solution a value of 3×10^8 M⁻¹ s⁻¹ was obtained.

At first sight, the reported result is in direct conflict with the many well established results on the *in vitro*²⁰ and *in vivo*^{21,22} sensitisation of chlorophyll fluorescence by carotenoids. Because many carotenoids exhibit only very weak fluorescence in solution, energy transfer was demonstrated by an indirect method—the excitation spectrum of the fluorescence of chlorophyll *a* in a chlorophyll– β -carotene mixture was shown to contain bands due to β -carotene²⁴. These experiments cannot,

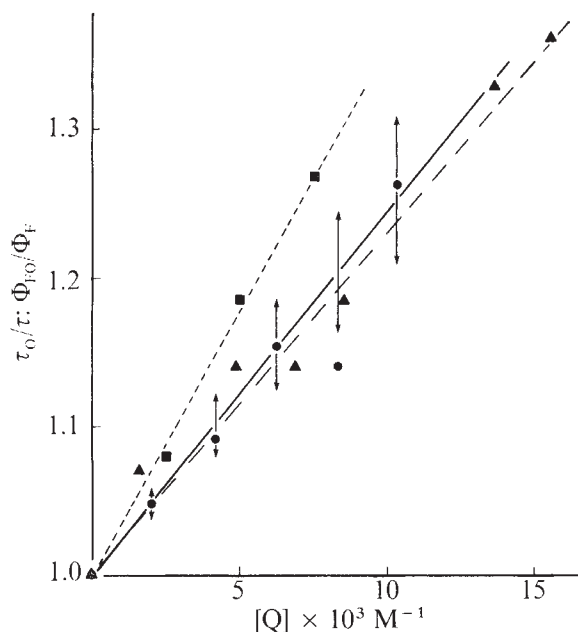
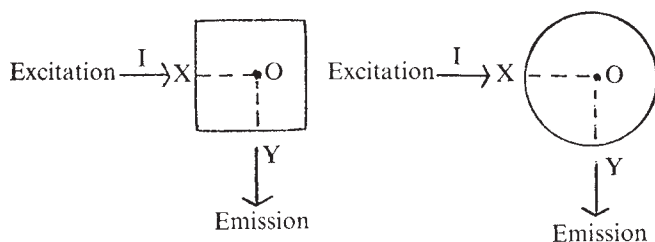


Fig. 1 Quenching of the fluorescence, and fluorescence lifetimes of chlorophyll *a* by β -carotene. Δ , τ_o/τ (— best fit K_{SV} 22.5); $\bullet$, Φ_{F0}/Φ_F (square cells, — best fit K_{SV} 26 $\pm$ 5); $\blacksquare$, Φ_{F0}/Φ_F (cylindrical cuvette — — — best fit K_{SV} 37 $\pm$ 5). τ and τ_o , Chlorophyll *a* lifetime in presence and absence of β -carotene. Φ_F and Φ_{F0} , Chlorophyll *a* fluorescence quantum yields in presence and absence of β -carotene. $[Q]$, Quencher concentration; K_{SV} , Stern-Volmer quenching constant. Φ_{F0}/Φ_F was calculated by the following procedure: consider the fluorescence from the centre O of a cell, illuminated at X by light of intensity *I*, and observed at right angles at Y.



In the absence of a quencher let the transmittance of the solution over XO and OY be x and y respectively. Then the fluorescence intensity at Y, $F_o = Ixy\Phi_{F0}$. If the transmittance of a solution of pure quencher over XO and OY is α and β respectively, then the new observed fluorescence intensity of quenched chlorophyll solution, $F_{obs} = Ixy\alpha\beta\Phi_F$. Hence the observed Stern-Volmer quenching $F_o/F_{obs} = \Phi_{F0}/\alpha\beta\Phi_F$. $\alpha = 10^{-\epsilon_1 c l}$ and $\beta = 10^{-\epsilon_2 c l}$; ϵ_1, ϵ_2 are extinction coefficients of β -carotene at excitation and monitored emission wavelength respectively; c is quencher concentration; l is distance from centre of cuvette to X and Y (normally equal).

because of their design, show whether the quenching process occurs.

We propose that the role of carotenoids in photosynthesis is wider than originally envisaged. On interaction of the excited singlet state of β -carotene with chlorophyll the energy can be partitioned between energy transfer and an electron transfer process. The relative importance of the two processes will be determined by the orientation and the distance separating the two molecules. Since energy transfer occurs by a resonance process^{20–22}, it should be favoured relative to the redox process by an increase in the distance separating the two molecules. The environment of carotenoids in the antennae is known to be different from that in the reaction centre^{24,25}. In the antennae resonance transfer from carotene to chlorophyll and rapid resonance transfer from one chlorophyll molecule to another may compete successfully against the electron transfer reaction such that only resonance transfer is occurring. At the reaction

centre, resonance energy transfer from the centre to a chlorophyll molecule outside the centre becomes inefficient because of the lower energy of the excited singlet state of the chlorophyll in the centres. Consequently, the electron transfer should be favoured and a chlorophyll radical anion and a carotene radical cation formed. This latter species will undergo a rapid ground state reaction with a neighbouring chlorophyll molecule^{22,26} to generate a chlorophyll radical cation²⁷ and thus the overall effect of the electron transfer reaction is to produce a charge separated pair $\text{Chl}^-\cdot\text{Chl}^+$. It has been proposed that such a dimer species is involved in photosystem II (ref. 25).

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Corrigendum

In the article 'Economics of alternative energy sources' by Martin Ryle, *Nature* **267**, 111–117, the figures given in Table 1 under Present Gas Turbine Generators should have included two entries: *a*, the contribution from oil-fired steam stations, showing an annual variation and *b*, the contribution from gas turbines used for peak boosting. These figures were inadvertently combined and used with the gas turbine time variations. The correct figures lead to a reduction in the final peak value in the Table from 187 to 163 GW and a reduction in the installed capacity needed from 266 to 233 GW; these figures are still some 50 times those of the present nuclear capacity.

matters arising

Culture and genetic variability

CHAKRABORTY found¹, by using gene frequencies for seven serological markers to compute genetic distance, that 'only geographical distance seems to be important, explaining the trend in genetic variabilities' in seven Chilean Indian populations. The correlation coefficients he presented were 0.716, 0.775, and 0.901, for correlations between genetic distance-geographical distance, genetic distance-cultural dissimilarity, and geographical distance-cultural dissimilarity, respectively. He then presented the results from a stepwise regression, and found that while geographical distance explained 51.31% of the genetic variation, cultural dissimilarity explained only an additional 8.88%. He did not, however, use as the first variable in the stepwise regression the one which had the highest correlation with the dependent variable. Culture alone accounts for 60.06% (r^2) of the genetic variation in these data. If geographical distance is then added, the additional variation explained would be less than 0.2%. These figures are, of course, approximations subject to slight rounding error from the published tables. It is indeed difficult to assess the relative importance of variables which are themselves highly correlated, as Chakraborty states. But his data clearly do not support his conclusion that of the variables measured, only geographical distance is an important correlate of genetic distance. From these data, culture is at least of equal importance.

It would be profitable to use these data to consider the relative effects of genetic and geographical distance upon culture. Geographical distance apparently explains 81.18% of cultural diversity as measured in this study. This leaves a smaller, but possibly significant, proportion of cultural variation which might be explained by the genetic measure.

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CHAKRABORTY REPLIES—Greene¹ contests my assertion regarding the relative importance of geographical versus cultural separation as a correlate of genetic variation in seven Chilean populations². In so doing, she notes that in the stepwise regression procedure I used geographic distance as the first variable even when the cultural dissimilarity index had a higher product moment correlation with genetic distance (the dependent variable). Two reasons justify my choice of geography as the first independent variable to be entered in the regression analysis, one being theoretical, the other pragmatic.

Take, for instance, cultural variability as the dependent variable. Evaluation of the relative effects of geographical and genetic distances on cultural variability indicate that 82.41% of the cultural variability is explained by geographical distance ($P < 0.0005$), whereas the additional effect of genetic distance is 4.01%, which is statistically insignificant ($0.10 > P > 0.05$). This alone may suggest that geographical distance should be the first variable of interest.

The pragmatic view concerns the choice of the characteristics determining cultural diversity. As elaborated elsewhere³, it is difficult to ascertain that cultural attributes are independent of geography. For example, we classified the seven populations on the basis of their types of subsistence, housing and architecture, economics, mode of gathering food, etc., which do have strong geographic components embedded in them. Thus, the strong association between cultural affinity and geographic proximity is indeed historical in these seven populations.

Furthermore, a stepwise regression method often under-represents the importance of the last variable to go in, particularly when the independent variables are significantly correlated with the dependent one. A logical explanation of causal relationship in such an event has to be based on an understanding of the process producing the covariation between the variables, and not based on which independent variable has the highest correlation with the dependent one.

Greene's criticism, therefore, should be viewed with caution, although any generality of my analysis needs further examination, possibly from studies of

other populations with comparable ethnographic accounts.

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- ¹ Greene, P. J. *Nature* 267, 375 (1977).
² Chakraborty, R. *Nature* 264, 350-352 (1976).
³ Chakraborty, R., Bianco, R., Rothhammer, F. & Llop, E. *Soc. Biol.* 23, 73-82 (1976).

Do viruses use calcium ions to shut off host cell functions?

IN discussing the shut-off of host cell functions by poliovirus, Carrasco and Smith¹ ask: "How does a viral protein change the ionic environment inside the cell?" Perhaps it forms channels across the external membrane². For example, a viral structural protein might generate holes by assembling into hexamers, pentamers, or extended lattices in a membrane.

Although Carrasco and Smith emphasised Na^+ ions, divalent ions are probably more important controllers of metabolism. In general, Ca^{2+} , Mg^{2+} and polyamine ions tend to form much more specific links with polyelectrolytes than Na^+ or K^+ do³, and the Debye-Hückel concept of ionic strength grossly underestimates the relative binding of, say, Ca^{2+} compared with K^+ , to membranes and viruses⁴. Many enzymic activities depend upon divalent ions, while relatively few respond to physiological changes in monovalent ion concentrations.

Of the two main divalent ions in biology, it is Ca^{2+} , not Mg^{2+} , that usually acts as a signal. This is because Ca^{2+} is actively pumped to at least a thousandfold concentration difference across membranes⁵, whereas Mg^{2+} is much nearer equilibrium⁶, probably because it permeates more easily. The fact that cells respond more to changes in extracellular Mg^{2+} than Ca^{2+} has misled some workers into putting a false emphasis upon the ions' relative roles⁷.

The early effects of many lytic viruses can readily be interpreted as a consequence of increased flow of Ca^{2+} into

¹ Chakraborty, R. *Nature* 264, 350-352 (1976).

cells. In some cases structural components of the virus perhaps act as the ionophores^{1,8,9}, while in others new proteins may need to be synthesised. The many phenomena observed in practice would then be merely the different facets of a fundamental gear-shift in polyelectrolyte-counterion interactions within the cell.

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CARRASCO AND SMITH REPLY—We find the proposal by Durham that changes in calcium ion concentration following infection by viruses are responsible for alterations in host cell functions very attractive. Our model to explain the shut-off of protein synthesis, which was briefly summarised in our *Nature* paper¹, and which is the subject of a more detailed paper², is in fact very similar. We also favour a model in which viral structural proteins form lattices in the cellular membrane and leave holes which act in the same way as some ionophores to allow the passage of ions².

We believe, however, that the shut-off of host cell protein synthesis is best explained by a change in Na⁺ ion concentration within infected cells, and we presented experimental evidence showing that sodium ions can have a differential effect on protein synthesis *in vitro* and indirect evidence that such changes in sodium ion concentration could occur *in vivo*¹. While we have not examined the effects of calcium ions in detail, a preliminary screening of the effects of many mono- and divalent cations on protein synthesis *in vivo* and *in vitro* failed to reveal a differential effect by calcium ions.

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Sodium emission in persistent meteor trains

USING theoretical estimates of the rate coefficient of the association of Na with O₃, Kolb and Elgin¹ have shown that the catalytic effect of sodium in releasing the store of recombination energy of free atmospheric atomic oxygen is more powerful than was previously assumed². Drawing on evidence from measured Na D-line intensities, Kolb and Elgin estimate a branching ratio for the production of excited Na(³P) from NaO reduction of $f \sim 0.05$, which, using the model previously suggested² implies that at 90 km sufficient photon emission occurs for an enduring train to result from a meteor of magnitude about -6. It is instructive to relate this estimate to observation. It is known that the trains of small duration show an initial emission decay at 90 km of about³ 0.2 mag s⁻¹ and since the radiation probably results from the interaction of ionic constituents of the train so that the reaction rate varies inversely as the meteor column cross-sectional area and hence inversely as time, a decay of 5 mag would be expected between about 1 s and 100 s. We may infer that any train luminosity having a duration $t \gtrsim 100$ s must be due to a catalytic (sodium) mechanism. In Olivier's catalogue⁴ 55% of trains ($t > 10$ s) were produced by high velocity shower meteors, Perseids, Orionids and Leonids. In the duration-magnitude characteristics given for 3700 trains by Millman⁵ the average meteor magnitude corresponding to a duration of 100 s is in the range -7.5 to -5 for velocities 60-70 km s⁻¹. From the extensive surveys of Hoffmeister (see ref. 4) and Olivier, one visual meteor in 780 results in a train of duration $t > 10$ s. Using ref. 5 the difference in magnitudes between meteors responsible for trains of 10 s and 100 s duration is 2.5 implying a corresponding incident meteor flux ratio⁶ of 27:1. Since the mean visual meteor rate⁶ to a single observer is 9.7 h⁻¹ the observed occurrence frequency of trains $t > 100$ s is 4.6×10^{-4} h⁻¹. In comparison the cumulative flux of meteors having magnitudes brighter than -6 for an individual observer is, using Hughes⁸ 3.1×10^{-4} h⁻¹. Though there are uncertainties in [O₃] and f and variations in Na abundance, the sodium cycle process is strongly supported as a source of persistent meteor train luminosity. This conclusion may be viewed in the light of the review of train characteristics by Hughes⁷.

Hughes considered evidence for the mechanisms responsible for persistent meteor train emission and on the basis of visual observations^{8,9} concluded that

the evidence supported a mechanism that is closely associated with the level of meteoric ionization rather than with meteoroid mass or meteor luminosity. It is of importance to emphasise that based on the observations^{8,9} the conclusion of Hughes is inappropriate. The results of Lindblad⁸ for Perseid meteor trains are confined to very short durations, t : for only 2% of the trains was $t > 3$ s while for 90% $t < 1.5$ s indicating that the observed light was associated with the meteor wake emission and in particular the well known OI 5577 Å feature. Indeed the data of Lindblad permit the determination of the effective lifetime τ (decay constant) of the emitting species. It is straightforward to show that the gradient of train duration-meteor magnitude plot is given by $-\tau(\log_e 10)/1.51$ and using Lindblad (Fig. 11B) $\tau = 0.21 \pm 0.05$ s. In comparison, the radiative lifetime of the OI (¹D₂ ← ¹S₀) transition is 0.74 s. However the great majority of observed trains were in the 90-100 km height interval (Lindblad, Fig. 18) where deactivation of the O(¹S₀) state most effectively occurs with O and O₂. Using recommended quenching coefficients and known atmospheric concentrations the quenching rate at 95 km is ~ 2.9 s⁻¹ resulting in an effective emission lifetime of 0.23 s. Plavec⁹ does not state the durations of trains in his analysis. We note, however, that according to Plavec (a), the trains disappeared in a few seconds, (b), there was concern with the effects of observer reaction times on duration measurements (c), 50% of Perseid meteors yielded trains in contrast to the much lower proportion at lower meteor velocities. The results indeed coincide with what is now known about the green line emission (the 5,577 Å feature was not identified until 1958). It is quite clear that the observations of Lindblad and Plavec do not refer to the true rare enduring train phenomenon.

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reviews

Thinking about complexity

Eric Ashby

Tools for Thought. By C. H. Waddington. Pp. 250. (Jonathan Cape: London, 1977.) £5.95.

HAL WADDINGTON did not live to see this book through the press. It is a fitting valedictory from one of the few of my contemporaries who deserve the epithet 'Renaissance Man'. Thirty-six years ago he published a little book called *The Scientific Attitude*, which delighted us by weaving poetry and art (with illustrations from Auden, Spender, Picasso and Paul Klee, among others) into a fabric of closely knit rational argument about science. For a generation he continued to carry out distinguished research in biology, and at the same time to publish thoughtful and stimulating work on ethical and social issues. In February 1975 he presided over an imaginative symposium at the Ciba Foundation, on *The Future as an Academic Discipline*. Over morning coffee he showed me the rough drafts of two books which were to embody his recent thinking. One draft was on *Man-Made Futures* (alas, not sufficiently advanced to be published posthumously); the other draft was *Tools for Thought*.

The title of the book accurately describes its content. Open your newspaper any morning and you'll read items such as these: conflict over transport policy for London; industrial dispute over the introduction of new equipment for typesetting; threat of a strike among sewage workers; apprehension about aerosols and ozone, or saccharine and cancer; alarm about population growth; warnings about depletion of resources; diplomatic hassles in the Middle East. We suffer saturation bombardment with advice about these issues, panaceas, utopian scenarios. What we rarely get is help on how to think about them. Waddington's book offers this help.

All these issues, and scores of others like them, have one property in common: they are embedded in a network of complexity. If doing *a* simply caused *b*, which in turn simply caused *c*, with no side-effects, how simple these issues would be to understand! But the tool

of linear thinking is useless on problems of complexity. It is well known that there are now new tools for thought—labelled systems analysis, information theory, decision theory, cybernetics, and so on. But most people who have not had to use these tools for professional purposes know very little about them and are put off by the turgid jargon in which they are packaged.

The purpose of Waddington's book is to provide a plain man's guide to these new tools for thought. It's not an instruction manual to teach you how to use them, but it demonstrates their capacities and their limitations, and it provides enough concrete examples to convince the plain man that what Waddington calls "Conventional Wisdom of the Dominant Group" (with the felicitous acronym COWDUNG) is often totally inadequate to handle contemporary social and technological problems.

After an introduction which identifies Waddington as a neo-Whiteheadian the book describes the conceptual tools for thinking about complex systems. First, it deals with shapes, with an illuminating digression on hierarchies and the use of matrices and Venn diagrams. It then deals with processes: a lucid exposition of exponentials and their consequences, sigmoid curves, feedback, chain reactions, the unexpected phenomena like schismogenesis discovered by Gregory Bateson, and an inkling of the way topologists describe complex social interactions by the use of catastrophe theory. After processes come communications, with a summary of some of the astonishing consequences of programming patterns into a computer. Communication leads to games theory and operational research, and this in turn to technological forecasting and system modelling (the one section of the book which I'm sure Waddington would have wished to revise if he had had the opportunity).

This is a catalogue of techniques daunting enough to repel the plain man for whom it is written. But my guess is that the plainest of men will find the book hard to put down. One of its delights is that it is so easy to read and

yet Waddington hasn't fudged any of the intellectual difficulties. The style is informal, conversational, as if the book had been taped direct from lectures. It combines a rare gift for popularisation with uncompromising scientific integrity; several times in the book Waddington as good as says: "I've shown you how this tool is used, but don't imagine you now know how to use it yourself. You don't." Just as the reader may be becoming puzzled at the description of some concept, Waddington throws in a specific example which dispels the puzzlement. Thus, in discussing the "prisoner's dilemma" (that theme so dear to philosophers and so ludicrous to the plain man), Waddington suddenly says that this is the paradigm for discussion about urban transport (I'll give up my car if everyone else gives up his car), grazing rights (I'll run fewer cows on the common if the others do the same), and the East-West arms race.

The book is illustrated by diagrams, most of which are deliberately imprecise because, as Waddington writes in the introduction, they are there to illustrate ideas, not facts. I see his point but I'm not happy about these diagrams. They are the sort of crude sketches you'd make on the blackboard while talking to a class. Some of them are insufficiently explained in the text. I may be pernickety, but in an otherwise elegantly printed book, I would like the lines marking the ordinates and abscissae of graphs to be straight. Graphs can be informal, but nevertheless tidy (as they are, for instance, in Hogben's *Mathematics for the Million*).

Hal Waddington was not able to correct the proofs of this book. Whoever did correct them ought to have taken more care. There are several misprints, some of them serious (for example, the date of Waddington's Bernal Lecture on p237). The diagram on p99 is upside down. I believe a diagram is missing altogether on p202, and possibly also on p220 or p221. The figure on p214 is inconsistent with the text. An *n* is missing from the equation on p207. Many of the diagrams could

be improved not only by a judicious use of a ruler to draw straight lines but by the addition of legends to supplement references in the text.

The book is, in my view, a brilliant essay for the plain man, particularly for the plain politician and administrator, who wants to understand how experts study complexity. It is a generous bequest from one of the most distinguished biologists of his generation. It deserves to be re-issued for a

wider public as a paperback at a moderate price; but it would be a courtesy to the memory of Hal Wadlington to re-edit the book conscientiously before it is re-issued. □

Lord Ashby was formerly Master of Clare College, Cambridge. He is Chancellor of the Queen's University, Belfast, and is at present Walgreen Professor of Human Understanding at the University of Michigan.

Mathematical treasure

Proofs and Refutations: The Logic of Mathematical Discovery. By Imre Lakatos. Edited by John Worrall and Elie Zahar. Pp. xii+174. (Cambridge University: Cambridge, London and New York, 1976.) Hardcover £7.50; paperback £1.95.

FOR anyone interested in mathematics who has not encountered the work of the late Imre Lakatos before, this book is a treasure; and those who know well the famous dialogue, first published in 1963–64 in the *British Journal for the Philosophy of Science*, that forms the greater part of the book, will be eager to read the supplementary material. The dialogue is what Lakatos calls a “rational reconstruction of history”, that is, the history of the Euler conjecture on polyhedra ($V-E+F=2$); the purpose is to illustrate principles of methodology. The participants seem themselves to be more interested in methodological questions than in the mathematical problem, which imparts a somewhat ponderous tone to the discussion; but the reconstruction, and the actual historical information given in the extensive footnotes, are in themselves fascinating. The supplementary matter, extracted by the editors of this posthumous work from earlier unpublished writings by Lakatos, and not revised by him before his death, consists, first, of a conclusion to the dialogue, in the form of a discussion of Poincaré’s proof, and, secondly, of much briefer case studies, not in dialogue form, of examples from analysis and measure theory.

The original dialogue, without this supplementary material, was stimulating but tantalising, in that it was unintended his readers to draw. Plainly, clear just what conclusions Lakatos one is a methodological thesis, concerning the process of mathematical discovery. According to Lakatos, the best model for this process is not the linear, Euclidean one of indubitable deductions from an initial set of axioms and

definitions, but that of an interplay between ideas for proofs and counter-examples in the light of which the proof is progressively improved. We begin, typically, with a conjecture and a tentative proof. We should then seek for counter-examples, guided by the proof: when these are found, we should not rule them out by *ad hoc* (“monster-barring”) definitions, even when it can plausibly be claimed that our original concepts, as we vaguely apprehended them, did not cover those examples; nor should we merely make an (“exception-barring”) restriction of the domain of application of the theorem so as to exclude the counter-examples. Rather, the proof must be re-analysed in the light of the counter-examples: in this way, we discover hidden lemmas, and build into the statement of the theorem the hypotheses essential for its validity.

With these maxims about how to do mathematics, few would disagree, despite a certain truculence in Lakatos’s manner of arguing for them. He has, however, another thesis, about how mathematics should be presented. He deplores the deductivist Euclidean style, in which a set of complicated definitions and axioms are stated at the outset, without explanation, and complex theorems follow, with their proofs in the barest form. Exposition in this manner conceals what is required for understanding: instead, it should reflect the actual history of the problems, “rationally reconstructed”, so that the motivation of the definitions, axioms and hypotheses of the theorems becomes apparent. This second thesis, about mathematical style, is more tendentious than the first, but has a great deal to be said in its favour. Certainly it impedes understanding when a definition is given without any illustration of the cases to which the term so defined does and does not apply, and without any explanation why it should be framed as it is; and certainly also the historical information embedded in most mathematical textbooks is often mythological. How much difference would be made to the best mathematical expositions by the adoption of

Lakatos’s recommendations depends on how much is allowable as “rational reconstruction”.

The most important question, however, is how far Lakatos intended to assert, as well as a methodological and a stylistic thesis, a *philosophical* one. Most people’s first reaction to Lakatos’s work is to say that the situation he describes is one likely to prevail in a branch of mathematics in its early stages, before it has attained the condition of an axiomatised theory, but not when it has reached maturity. On this view, Lakatos’s observations have no philosophical consequences: they describe how we do, or can best, arrive at a developed theory, how we can make mathematical discoveries, not what such a theory is like when we have succeeded in framing it. There are, however, passages (pages 100 and 138) suggesting that Lakatos took a quite different view, namely that the process of finding counter-examples and re-analysing proofs in the light of them is never-ending, or, at least, that we can never know that we have reached an end.

This thesis, if accepted, would indeed be of critical philosophical importance, since it would demand, though it would not itself provide, a revision of most of the known accounts of mathematical knowledge, if not of mathematical truth. Other passages (for example, p. 124), however, suggest that the process does terminate. The editors are more clearly of this opinion than Lakatos himself (pages 125–6 and 138n). They think that, by formalisation, one can dispel all doubt from the proof, and throw it upon the axioms: from these, however, it can never be removed. This leaves a big lacuna in the whole discussion: the character of doubt concerning the axioms of a mathematical theory is far from obvious. Thus, in the upshot, we are presented, in this book, with a piece of work which, although admirable as a historical study, is, from a *philosophical* standpoint, incomplete and inconclusive. Doubtless the delay in publication was due to Lakatos’s awareness of this: had he lived, we should presumably have eventually had a more finished work.

Nevertheless, the book, as it stands, is rich and stimulating, and, unlike most writings on the philosophy of mathematics, succeeds in making excellent use of detailed observations about mathematics as it is actually practised.

Michael Dummett

Michael Dummett was Reader in the Philosophy of Mathematics at the University of Oxford from 1961–74, and has been Senior Research Fellow at All Souls’ College, Oxford, UK, from 1974.

Ramifying phylum

The Biology of the Mollusca. By R. D. Purchon. (Second edition.) Pp xxv+560. (Pergamon: Oxford and New York, 1977.) £17.50.

ARISING from a common stock with the segmented annelids and arthropods, the molluscs proceeded to evolve along totally different lines, to attain in the cephalopods the culmination of invertebrate organisation. They did so from a basis of possibly secondary simplicity—this depending on whether or not the enigmatic *Neopilina* may be regarded as segmented.

Contemplation of the structure of early fishes might have led some contemporary evolutionist to predict developments in structural pattern that could lead to amphibious and eventually terrestrial success. Similar survey of the first obscurely crawling molluscs could have afforded no faintest indication of possibilities eventually realised in the torsionally produced gastropods, compressed and insinuating bivalves, and predacious, rapidly darting squids.

The consequences of the radiating evolutionary possibilities of primitive molluscan structure are admirably revealed in this second edition of a book which received all too little notice when first published in 1968.

Although a sizeable volume it cannot hope to cover the enormous extent of form, function and environmental

specialisation achieved by members of this intricately ramifying phylum, with convergence running wild to produce both snail-like bivalves and bivalve gastropods. The author has concentrated on groups or aspects with which he is most at home or which he feels are of greatest significance.

Almost complete adherence to the original pagination has prevented any extensive introduction of new material. Due note has, however, been taken of the recent work of Professor J. A. Allen on the Verticordiidae. These latter bring the septibranch bivalves into unquestioned association with the eulamellibranchs and exclude previous ideas about possible direct connection with the protobranchs.

The precise nature and course of the digestive processes in the bivalves continues to provoke investigation. Here, Professor Purchon provides a judicious review of the present state of the controversy, largely stimulated by the work of Dr Brian Morton, on the precise cyclic nature of these processes. He has also brought up to date his sections on the distribution, and mode of spreading, of gastropods on Pacific islands.

This new edition provides an admirable introduction to the advanced study of this significant phylum.

C. M. Yonge

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh, UK.

Fusion research

Nuclear Fusion. Special Supplement, 1976. (World Survey of Major Facilities in Controlled Fusion Research.) Pp. 866. (International Atomic Energy Agency, Vienna, 1976.) \$48.

THIS large volume provides a unique insight into the magnitude and variety of the world's research and development effort in nuclear fusion. Following the false dawn at the time of the early Geneva Conferences, an immense amount of experimental and theoretical work by the plasma physicists has led to the construction of numerous plasma-confining devices, quite apart from more recent initiatives on the entirely distinct concept of laser-implosion devices. Now several laboratories, notably Argonne and Oak Ridge in the United States have embarked on long-range (20 years plus) development programmes of operating fusion reactors on the basis of the tokamak confinement principle, incorporating detailed development programmes in all the subsidiary technologies from ultrahigh power electrical supplies to tritium processing.

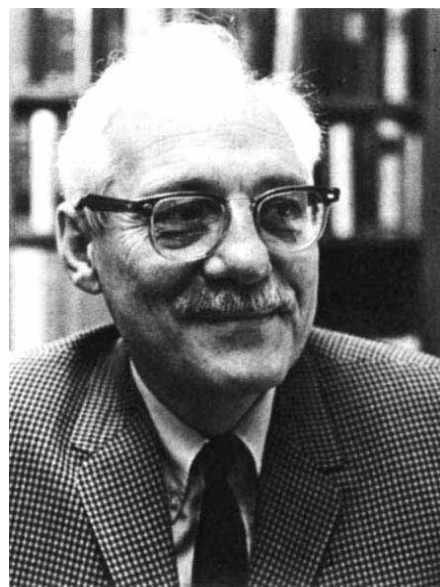
The World Survey first classifies programmes by country and laboratory; each entry specifies purpose, key personnel, main features, major results and future plans. Numerous perspective drawings of actual or conceptual reactor designs are included, the Americans, Russians, Japanese and Germans seeming to have the largest and most varied programmes. There follows a summary section classified by types of programme (confinement systems, plasma physics, heating techniques and fuel injection strategies, reactor considerations including materials research, and laser research—including laser-aided fuel injection into plasmas). There is an alphabetical index of more than 3,000 key scientific workers.

It is a major achievement to have assembled so much solid information in standardised format from so many sources. The scale and scope of the work described implies the growing optimism that nuclear fusion represents a genuine hope as an energy source for the twenty-first century.

Robert W. Cahn

Robert W. Cahn is Professor of Materials Science at the University of Sussex, UK.

The May issue of **Nucleic Acids Research** is devoted to papers written by former colleagues and friends of Jerome Vinograd (1913–1976). This memorial issue is respectfully dedicated to him.



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Population analysis

Spatial Population Analysis. By P. H. Rees and A. G. Wilson. Pp. 356. (Arnold: London; Academic: New York, 1977.) £27.50; \$35.50.

ALL would agree with the authors' aim to provide good methods of population analysis for effective social and economic planning; this detailed and thorough text provides such an essential basis. In their preface, the authors warn the reader that he must face considerable conceptual difficulties in acquiring the skill, but comfort him by claiming relatively little demand on formal mathematical knowledge apart from algebra and an elementary knowledge of matrices. Nevertheless, this is a formidable text that needs to be painstakingly worked through to follow the argument.

The strength of the text is claimed to be that for the first time the analysis takes full cognisance of migration through the "use of new and powerful conceptual and mathematical tools". Such recognition of the importance of migration is welcome, since it has received scant treatment in some recently published work, a strange weakness in a world of growing mobility.

The text itself is arranged in five parts, each well introduced, and the argument subsequently explained and documented. The first part introduces current population models and their associated problems: here the authors introduce one of their fundamental features—models based on age groups and not life-tables, a basic divergence from the work of Keyfitz on the mathematics of population. It is also pointed out that although concentration is on theoretical concepts, examples using real data are introduced to illustrate the argument.

The second part introduces the concept of "population accounting" and is illustrated by reference to aggregate populations. The third part is devoted to the development of realistic population models, followed in the fourth part by the putting forward of the concept of life-table accounts to demonstrate that spatial dimensions may be added to conventional life-table analysis to make associated models more systematic, consistent and rigorous. The final, fifth, part summarises the new methods and their applications as well as their relationship to development in other fields.

The authors have been careful to make sure their work can be applied empirically by limiting themselves to commonly available data, although

they have interwoven their own work with that of Lexis, Pressat, Rogers, Stone and others already accepted among demographers.

With page after page of mathematical formulae and matrices, the publishers have faced a monumental task in producing the book in present conditions at a realistic price. It is no wonder that the book is nevertheless expensive.

R. E. H. Mellor

R. E. H. Mellor is Professor of Geography at the University of Aberdeen, UK.

γ -ray astronomy

Gamma-Ray Astronomy. By E. L. Chupp. (Reidel: Dordrecht, The Netherlands and Boston, Massachusetts, 1976.) Cloth Dfl 105; paper Dfl 50.

THE title on the cover of this book reads *Gamma-Ray Astronomy* but inside a subtitle, *Nuclear Transition Region* has been added. This uncertainty as to the scope of the book extends into the text and gives an uneven quality to what is, otherwise, a valuable contribution to this field of astronomy.

The first chapter is a brief introduction and it is followed by a chapter on the fundamental mechanisms which produce γ rays. Those processes which lead to line spectra, such as transitions between excited states of nuclei and the annihilation of positrons, are treated very thoroughly. Presumably for the sake of completeness this chapter also deals with mechanisms such as Compton scattering, *bremsstrahlung* and the decay of neutral pions, all of which lead to the production of continuous spectra.

The mechanisms discussed in chapter 2 are then used in the next chapter to predict the γ -ray emission which may be expected from astronomical sources. The inclusion of a chapter on predicted fluxes was perhaps necessary because of the paucity of experimental data in γ -ray astronomy, but this does lead to a certain amount of repetition in the book because some of the discussions are presented again when the experimental results are described in chapter 5. The author's interest in continuous spectra seems to waver a little in chapter 3 and he provides only a brief discussion of the high energy γ -ray emission from pulsars and supernova remnants, and makes no mention of extragalactic sources such as radio galaxies and quasars. Chapter 4 is concerned with the interactions of γ rays with matter and is included as an introduction to

experimental problems in γ -ray astronomy.

Chapter 5 is devoted to observational results. As in chapter 3, the treatment of the sources of continuous spectra is not consistent; for example, no mention is made of the many measurements of the pulsar NPO532 in the Crab nebula although the unsuccessful searches for γ rays from extragalactic supernovae are described.

Chapter 6 deals with experimental problems in γ -ray astronomy and, appropriately, is the longest chapter in the book. It contains a comprehensive discussion of the background problems in a wide variety of detectors; if the quantity of data referred to does seem a little forbidding to the newcomer to the field, it will at least remind him of the need for technical innovation if this branch of astronomy is to make significant progress.

This is the first book to place the correct emphasis on the experimental problems in γ -ray astronomy and will therefore be invaluable both to the newcomer and to the experienced worker in this field. **R. R. Hillier**

R. R. Hillier is Lecturer in the Department of Physics, University of Bristol, UK.

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announcements

On the move

S. K. Banerjee, Professor of Geology and Geophysics at the University of Minnesota, is on sabbatical leave from 1 September 1977 to 30 June 1978, as a Visiting Scholar in the Office for History of Science and Technology, University of California, Berkeley, and a Visiting Professor in Geophysics at both Stanford University and U.C. Berkeley. Communications to 722 Wildcat Canyon Road, Berkeley, CA 94708.

A. Albert, Research Professor at the State University of New York at Stony Brook, to Emeritus Professor in the Research School of Chemistry, Australian National University, Canberra, P.O. Box 4, A.C.T. 2600, Australia.

Appointment

Professor J. G. Beetlestone, to the recently created Chair of Science Education in the Department of Education at University College, Cardiff.

Members of the National Academy of Sciences elected on 26 April 1977

G. A. Almond, Stanford University. **M. Artin**, Massachusetts Institute of Technology. **F. E. Bloom**, The Salk Institute for Biological Studies, San Diego, California. **W. S. Broecker**, Columbia University. **H. Brown**, Secretary of Defense, Washington, DC. **P. W. Choppin**, Rockefeller University. **R. K. Clayton**, Cornell University. **E. F. Colson**, University of California at Berkeley. **J. M. Dawson**, University of California at Los Angeles. **G. Debreu**, University of California at Berkeley. **T. O. Diener**, Agricultural Research Service, US Department of Agriculture. **S. Epstein**, California Institute of Technology. **H. E. Evans**, Colorado State University. **W. Feit**, Yale University. **S. L. Glashow**, Harvard University. **G. Goldhaber**, University of California at Berkeley. **J. E. Gunn**, California Institute of Technology. **N. B. Hannay**, Bell Laboratories, Inc., Murray Hill, New Jersey. **D. Harker**, State University of New York, Buffalo. **W. R. Hewlett**, Hewlett-Packard Company, Palo Alto, California. **G. H. Hitchings**, Burroughs Wellcome Fund Research Laboratories, Research Triangle Park, North Carolina. **L. N. Howard**, Massachusetts Institute of Technology. **J. D. Jennings**,

Person to Person

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Furnished five bedroom house in Villebon-sur-Yvette, near Orsay, Faculté de Sciences, Université de Paris-XI, France, offered in exchange for at least 3 bedroom house in Frankfurt-am-Main, Germany, for August 1977. Villebon is next to Orsay, where the Faculté de Sciences of the University of Paris-XI is located. It is about 20 km south-west of Paris. Please contact Dr Francisco Alvarado, Comparative Physiology, Université de Paris-XI, 91405, France, for further information.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

University of Utah. **N. Keyfitz**, Harvard University. **E. D. Kilbourne**, Mount Sinai School of Medicine. **M. J. Klein**, Yale University. **R. M. Krause**, National Institutes of Health. **I. R. Lehman**, Stanford University. **S. Lieberman**, Columbia University. **R. S. Lindzen**, Harvard University. **J. W. Littlefield**, The Johns Hopkins University. **H. O'Neill McDevitt**, Stanford University Hospital. **A. A. Moscona**, University of Chicago. **E. F. Neufeld**, National Institutes of Health. **O. E. Neugebauer**, Brown University. **R. M. Noyes**, University of Oregon. **S. L. Palay**, Harvard Medical School. **R. P. Perry**, University of Pennsylvania. **J. C. Phillips**, Bell Laboratories, Inc., Murray Hill, New Jersey. **G. W. Preston III**, Hale Observatories, Pasadena, California. **W. van Orman Quine**, Harvard University. **P. H. Raven**, Missouri Botanical Garden, St Louis. **C. N. Reilly**, University of North Carolina. **H. Reiss**, University of California at Los Angeles. **B. Richter**, Stanford Linear Accelerator Center, Stanford University. **R. Sager**, Harvard Medical School. **L. H. Sarett**,

Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey. **R. N. Shepard**, Stanford University. **P. S. Skell**, Pennsylvania State University. **D. Slepian**, Bell Laboratories, Inc., Murray Hill, New Jersey. **H. Tabor**, National Institutes of Health. **J. H. Taylor**, Florida State University. **R. F. Thompson**, University of California at Irvine. **G. R. Tilton**, University of California at Santa Barbara. **S. C. C. Ting**, Massachusetts Institute of Technology. **W. N. Valentine**, University of California at Los Angeles. **E. M. Witkin**, Douglass College, Rutgers University. **G. N. Wogan**, Massachusetts Institute of Technology. **J. Wolpert**, Princeton University. **H. E. Wright, Jr.**, University of Minnesota, Minneapolis.

Awards

The Ciba-Geigy Drew Award in Biomedical Research (\$2,000) will be granted annually. The topic for the initial awards and symposium is Viral Etiology of Cancer and the recipients are Robert C. Gallo, and Fred Rapp. The first symposium will be held on 20 October 1977.

The André Lichtwitz prize (9,500 French francs) is awarded every year by INSERM for French or foreign scientists (or a team) who have specially distinguished themselves in research on calcium and phosphorus metabolism carried out during the previous year, whether in the clinical or more fundamental fields. Conditions of entry for the 1977 prize are obtained from INSERM. Applications must reach the Director of the INSERM (c/o Mlle. C. Chirol, 101 rue de Tolbiac, 75645-Paris Cedex 13, France) by 30 June 1977.

The 'Life Institute' prize (EDF Foundation), of 300,000 F, was shared by a Japanese research team represented by Makio Ushida, and a Swedish chemist, Sören Jensen. The international jury, rewarded the Japanese for their work on the toxicity of methyl mercury, which is the cause of Minamata Disease. Sören Jensen was rewarded for his work on the polychlorobiphenyls. These organic derivatives are used in paints, lubricants, rubbers and so on. As they are fat-soluble and are not biodegradable they tend to accumulate and may contaminate food processing plants.

Vacancies

Two vacancies exist in the International Commission on Zoological Nomenclature owing to resignations. Nominations for candidates for election to the vacancies should be sent to the Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, United Kingdom, within three months of the date of publication of this notice in the *Bulletin of Zoological Nomenclature*. Candidates must be eminent scientists, irrespective of nationality, with a distinguished record in any branch of zoology, who are known to have an interest in zoological nomenclature.

Nominations must state the name, date of birth, nationality, field(s) of specialisation and qualifications of each candidate, and the name(s) and status of the nominator(s). A list of the candidate's publications and his *curriculum vitae* would also be helpful.

Reports and Publications

Other countries—March

- CERN—European Organization for Nuclear Research. CERN 77-05: A New Warning System for Fires of Electrical Origin. By A. H. Pietersen. Pp. v+5. (Geneva: CERN, 1977.) [283]
- Smithsonian Contributions to Paleobiology, No. 30: Triassic Echinoids. By Porter M. Kier. Pp. iv+88. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [283]
- Solid State Physics in the People's Republic of China. Edited by Anne Fitzgerald and Charles P. Slichter. (A Trip Report of the American Solid State Physics Delegation. CSCPRC Report No. 1.) Pp. xiii+203. (Washington, DC: National Academy of Sciences, 1976) \$10.25. [283]
- New Zealand: Department of Scientific and Industrial Research. Botany Division Triennial Report, 1972/1975. Pp. 84. (Christchurch: Department of Scientific and Industrial Research, 1976.) [283]
- Institutt for Atomenergi, Kjeller. Kjeller Report 154: Atmospheric Deposition of Trace Elements in Norway Studies by Means of Moss Analysis. By E. Steinnes. Pp. 13+3 tables+10 figures. (Kjeller, Norway: Institutt for Atomenergi, 1977.) [283]
- Tracor Inc. 1976 Annual Report. Pp. 32. (Austin, Texas: Tracor, Inc., 1977.) [293]
- European Brewery Convention. Reports of the Barley Committee. Field Trials 1973 (Malting 1974). Pp. viii+155. Field Trials 1974 (Malting 1975). Pp. viii+160. Field Trials 1975 (Malting 1976). Pp. viii+163. (Vols. 24, 25 and 26.) (8050 Freising-Weihenstephan, West Germany: L. Reiner, EBC-Databank, Techn. Univ. München-Ackerbau und Versuchswesen, 1974, 1975 and 1976.) [303]
- Bulletin of the American Museum of Natural History, Vol. 158, Article 3: A Synopsis of Asian Species of *Mus* (Rodentia, Muridae). By Joe T. Marshall, Jr. Pp. 173-220. (New York: American Museum of Natural History, 1977.) \$2.90. [303]
- Climate and Food: Climatic Fluctuation and U.S. Agricultural Production. Pp. ix+212. (Washington, DC: National Academy of Sciences 1976.) \$7.75 [303]
- World Health Organization. Technical Report Series, No. 602: Advances in Viral Hepatitis—Report of the WHO Expert Committee on Viral Hepatitis. Pp. 62. (Geneva: WHO; London: HMSO, 1977.) \$r.fr.8; \$3.20. [313]
- United States Department of the Interior: Geological Survey. Professional Paper 971: Structural Geology of the Confusion Range, West-Central Utah. By Richard K. Hise. Pp. iii+9+plate 1. (Washington, DC: US Government Printing Office, 1977.) [313]
- CERN—European Organization for Nuclear Research. CERN 77-04: The $\pi\pi$ Interaction. By J. L. Petersen. Pp. 109. (Geneva: CERN, 1977.) [313]

UK & Ireland—April

- Nonlinear Analysis: Theory, Methods and Applications*, Vol. 1, No. 1, 1976. Edited by V. Lakshmikantham. Pp. 1-90. Annual Subscription Rates for 1977: For libraries, University Departments, Government Laboratories, Industrial and other multiple-reader institutions US\$8. Individual whose institution takes out a library subscription may purchase a second or additional subscriptions for personal use US\$30. (Oxford and New York: Pergamon Press, 1977.) [444]
- Monitoring and Assessment Research Centre, Chelsea College, University of London. MARC Report No. 1: The Ozone Depletion Problem (An example of

Harm Commitment). By Lester Machta. (A General Report.) Pp. 33. £1; \$2. MARC Report No. 2: Vanadium in the Environment. By Siv Bengtsson and Germund Tyler. (A Technical Report.) Pp. 17. £1; \$2. MARC Report No. 3: Suggestions for the Development of a Hazard Evaluation Procedure for Potentially Toxic Chemicals. By Robert C. Harris. (A Research Memorandum.) Pp. 18. £1; \$2. (London: Monitoring and Assessment Research Centre, Chelsea College, 1977.) [444]

Advances in Extractive Metallurgy. (An International Symposium organized by the Institution of Mining and Metallurgy and held in London from 18 to 20 April, 1977.) Pp. vii+244. (London: The Institution of Mining and Metallurgy, 44 Portland Place, W1, 1977.) £18.50. [444]

National Radiological Protection Board. NRPB-R49: Fallout in Rainwater and Airborne Dust-Levels in the UK during 1975. By G. J. Hunt, B.M.R. Green and D. Elliot. Pp. 15. 50pp. NRPB-R50: Technical Specification for the NRPB Fast Neutron Personal Dosimeter. By D. T. Bartlett. Pp. 3. 50p. (Harwell, Didcot, Oxon: NRPB, 1976. Available from HMSO, London.) [444]

British Industry Today: Energy. Pp. 30+8 photographs. (London: HMSO, 1977.) 90p net. [444]

Loughborough University of Technology: Department of Civil Engineering. Third International Conference on Environmental Health Engineering in Hot Climates and Developing Countries: Planning for Water and Waste in Hot Countries, 26th-28th September 1976—Proceedings. Edited by John Pickford. Pp. 110. (Loughborough, Leics: Rowena Steele, Department of Civil Engineering, University of Technology, 1977.) £5. [444]

Department of the Environment. Welsh Office. Accommodation of Gypsies. (A report on the working of the Caravan Sites Act 1968, presented to the Secretary of State for the Environment, December 1976.) Pp. viii+50. (London: HMSO, 1977.) £1 net. [54]

The Zoological Society of London. Annual Report 1976. Pp. 52. (London: The Zoological Society of London, 1977.) [54]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 278, No. 959: A Discussion on Water Structure and Transport in Biology. Organized by R. E. Richards, FRS, and F. Franks. Pp. 1-205+5 plates. UK £12.95; Overseas £13.20. Vol. 278, No. 960: An Anomaly in the Response of the Eye to Light of Short Wavelengths. By J. D. Mollon and P. G. Polden. Pp. 207-240. UK £2.05; Overseas £2.15. (London: The Royal Society, 1977.) [64]

Preventing Bronchitis. Pp. 40. (London: Office of Health Economics, 162 Regent Street, W1, 1977.) 35p. [74]

Field Studies Council. Annual Report 1975/1976. Pp. 40. (London: Field Studies Council, 9, Devereaux Court, Strand, WC2, 1977.) [74]

Albright and Wilson, Ltd., Annual Report 1976. Pp. 36. (London: Albright and Wilson, 1 Knightsbridge Green, SW1, 1977.) [74]

Mineral Resources Consultative Committee. Mineral Dossier No. 17: Sandstone. Compiled by P. M. Harris. Pp. v+37. (London: HMSO, 1977.) £1.50 net. [74]

University of Cambridge. Annual Report of the Department of Geodesy and Geophysics, 1975/1976. Pp. 8. (Cambridge: The University, 1976.) [124]

Proceedings of the Royal Irish Academy. Vol. 76, Section A, No. 20: On the Theory of Dielectric Saturation of Polar Fluids. By W. T. Coffey and B. K. P. Scaife. Pp. 195-216. (Dublin: Royal Irish Academy, 1976.) £1.85. [124]

Potato Marketing Board. Sutton Bridge Experimental Station: Annual Review—1976. Pp. 78. Summary of Investigations Undertaken in 1975/76. Pp. 4. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1977.) gratis. [134]

Proceedings of the Royal Irish Academy. Vol. 76, Section A, Nos. 21-23: Symposium on Harmonic Analysis and Topological Algebras (December 1975). Pp. 217-342 £5.80. Vol. 77, Section B, No. 1: Littoral and Benthic Investigations on the West Coast of Ireland—VI. By J. K. Partridge. Pp. 1-64. £2.16. (Dublin: Royal Irish Academy 1976 and 1977.) [254]

The Ciba Foundation. Annual Report 1976. Pp. 48. (London: The Ciba Foundation, 1977.) [254]

Science Museum Library. Science Library Bibliographical Series No. 803: Some References on Practical Arithmetic in Western Europe in the 13th-15th Centuries. Pp. 10. (London: Science Museum Library, 1976.) [254]

Pollution Research and the Research Councils. Pp. 61. The Report of a Working Group on Ecological Research on Seabirds. Pp. iv+48. (London: Natural Environment Research Council, 1977.) [254]

Daresbury 1976. Pp. 66. (Daresbury, Warrington: Science Research Council, Daresbury Laboratory, 1977.) [254]

Thames Migratory Fish Committee—Report. Pp. 40. (London: Thames Water, New River Head, Rosebery Avenue, EC1, 1977.) [254]

Health and Safety Commission. Hazardous Substances (Conveyance by Road), Tank Labelling Regulations 1977, and Transport Hazard Information Rules. (Consultative Document) Pp. 30. (London: Enquiry Point, Health and Safety Executive, Baynards House, 1 Chepstow Place, W2, 1977) gratis [154]

Department of Industry, Technology and the Environment. Reports from Scientific Counsellors Overseas, No. 10: Water. Pp. ii+66. (London: Department of Industry, Abell House, John Islip Street, SW1, 1977.) [274]

Fisons Limited. Report and Accounts for the year ended 31 December 1976. Pp. 32. (London: Fisons Limited, 9 Grosvenor Street, W1, 1977.) [284]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 278, No. 961:

A Discussion on Structural and Functional Aspects of Plasticity in the Nervous System. Organized by H. B. Barlow, FRS and R. M. Gaze, FRS. Pp. 241-436+22 plates. (London: The Royal Society, 1977.) UK £15.30; Overseas £15.80. [284]

Other countries—April

Energy: The Solar Prospect. By Denis Hayes. (Worldwatch Paper No. 11.) Pp. 79. (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, NW, 1977.) \$2. [444]

Studia Forestalia Suecica. Nr. 135: Flowering in a Seed Orchard of *Pinus sylvestris* L. By Alena Jonsson, Inger Ekberg and Gösta Eriksson. Pp. 38. Nr. 136: The Effect of Inorganic Nutrients on Water Economy and Hardness of Conifers II. By Lars Christersson. Pp. 23. (Stockholm: Skogshögskolan, Royal College of Forestry, 1976.) [444]

World Health Organization. Technical Report Series, No. 601: Methods Used in Establishing Permissible Levels in Occupational Exposure to Harmful Agents—Report of a WHO Expert Committee with the participation of ILO. Pp. 68. Sw.fr.8; \$3.20. No. 602: Advances in Viral Hepatitis—Report of the WHO Expert Committee on Viral Hepatitis. Pp. 62. Sw.fr.8; \$3.20. (Geneva: WHO; London: HMSO, 1977.) [444]

The British National Health Service: Conversations with Sir George Godber. (A Publication of the Geographic Health Studies Program, John E. Fogarty International Center for Advanced Study in the Health Sciences.) Pp. ix+159. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1977. For sale by US Government Printing Office, Washington, DC.) \$1.80. [444]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15383: Effects of Noise on Man. By G. J. Thiessen. Pp. 89. (Ottawa: National Research Council Canada, 1976.) \$2.50. [444]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2037: Hydrologic Changes After Logging in Two Small Oregon Coastal Watersheds. By D. D. Harris. Pp. vi+31. (Washington DC: US Government Printing office, 1977.) [54]

Development of Social Sciences and Science Policy Studies in Europe. By Jean Jacques Salomon. (Lecture Series-2/76). Pp. 47. (New Delhi: Centre for the Study of Science, Technology and Development, Council of Scientific and Industrial Research, 1976.) [54]

Bulletin of the American Museum of Natural History. Volume 158, Article 2: Review of Ichthyofauna and Other Mesozoic Teleost Fishes and the Theory and Practice of Classifying Fossils. By Colin Patterson and Donn Eric Rosen. Pp. 81-172. (New York: American Museum of Natural History, 1977.) \$4.95. [54]

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Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Mechanical Engineering, 1974/1975. Pp. 50. (Melbourne: CSIRO, 1976.) [74]

Diving/Dykning, Vol. 1, No. 1, 1977. Pp. 1-20. Edited by George Ballantine. (Soebay, Aerøe Island, Denmark: Association of Danish Divers, 1977.) [74]

World Health Organization. Alcohol-Related Disabilities. Edited by G. Edwards, M. M. Gross, M. Keller, J. Moser and R. Room. (WHO Offset Publication No. 32.) Pp. 154. (Geneva: WHO; London: HMSO, 1977.) Sw.fr.18; \$7.20. [74]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Marine Biochemistry Unit 1975-76, July 1-June 30. Pp. 48. Annual Report of the Division of Textile Physics, 1975-76. Pp. iii+41. (Sydney: CSIRO, 1976.) [74]

Smithsonian Contributions to Zoology, No. 246: Freshwater Triclad (Turbellaria) of North America, IX: The Genus *Sphalloplana*. By Roman Kenk. Pp. iii+38. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [124]

Cretaceous-Tertiary Extinctions and Possible Terrestrial and Extraterrestrial Causes. By the L-TEC Group. (Proceedings of the Workshop held in Ottawa, Canada, 16-17 November 1976.) Pp. 162. (Ottawa: National Museums of Canada, 1977.) [124]

The National Health System in Denmark: A Descriptive Analysis. By D. Gannik, E. Holst and M. Wagner. (Publication of The John E. Fogarty International Center for Advanced Study in the Health Sciences, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health.) Pp. vi+86. (Washington, DC: US Government Printing Office, 1977.) \$1.15. [124]

United States Department of the Interior: Geological Survey. Bulletin 1420: Mineral Resources of the La Garita Wilderness, San Juan Mountains, Southwestern Colorado. By Thomas A. Steven and C. L. Bieniewski. (Studies Related to Wilderness—Wilderness Areas.) Pp. vi+65+1 plates. Professional Paper 989: Plutonism and Orogeny in North-Central Washington—Timing and Regional Context. By Kenneth F. Fox, Jr., C. Dean Rinehart and Joan C. Engels. Pp. iii+27. (Washington, DC: US Government Printing Office, 1977.) [124]

World Health Organization. Technical Report Series, No. 603: Engineering Aspects of Vector Control Operations—First Report of the WHO Expert Committee on Vector Biology and Control. Pp. 40. Sw.fr.6. Oral Health Surveys: Basic Methods. Second edition. Pp. 68. Sw.fr.12; \$4.80. Lists of International Biological Standards, International Biological Reference Preparations, and International Biological Reference Reagents, 1977. Pp. 72. Sw.fr.12; \$4.80. (Geneva: WHO; London: HMSO, 1977.) [124]